






# Implementation of rapid diagnostics assays for detection of histoplasmosis and cryptococcosis in central american people living with HIV

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## Abstract

**Objectives:** Histoplasmosis and cryptococcosis are important public health problems in people living with HIV (PLHIV) in Central America. Conventional laboratory assays, based on microscopy and culture, are not optimal for the diagnosis of either disease. However, antigen (Ag) assays are rapid and highly accurate for the diagnosis of these infections.

**Methods:** Laboratory surveillance of PLHIV was carried out in four hospitals in Panama, Honduras and Nicaragua, between 2015 and 2019. Detection of *Histoplasma* antigens in urine was performed by enzyme immunoassay (EIA), and *Cryptococcus* antigen detection in sera and cerebrospinal fluid specimens was performed by lateral flow assay (LFA).

**Results:** A total of 4,453 PLHIV with clinical suspicion of histoplasmosis ( $n = 1,343$ ) or cryptococcosis ( $n = 3,110$ ; 2,721 sera and 389 CSF) were tested. Of 1,343 patients suspected of having histoplasmosis, 269 (20%) were *Histoplasma* Ag positive. Of 3,110 patients tested using the *Cryptococcus* Ag assay, 329 (11%) were positive. Honduras reported the highest positivity rates (32% for *Histoplasma* Ag, and 16% for *Cryptococcus* Ag); Panama reported the largest number of patients testing positive using the *Histoplasma* Ag assay ( $n = 201$ ); and Nicaragua reported the largest number of patients testing positive using the *Cryptococcus* Ag assay ( $n = 170$ ).

**Conclusion:** Here, we show how the implementation of rapid diagnostics assays impacted case detection and was useful for the care of people with advanced HIV. Rapid and accurate diagnosis could reduce mortality associated with histoplasmosis and cryptococcosis in PLHIV.

## KEYWORDS

AIDS, cryptococcosis, *Cryptococcus*, *Histoplasma*, histoplasmosis, HIV

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## 1 | INTRODUCTION

People living with HIV (PLHIV) with advanced diseases are at high risk of developing multiple opportunistic infections including histoplasmosis and cryptococcosis.<sup>1-4</sup> Clinical signs and symptoms of these infections are often non-specific and therefore difficult to accurately diagnose and differentiate from other infections caused by non-fungal pathogens (eg tuberculosis).<sup>5-7</sup> Conventional laboratory methods for diagnosing histoplasmosis and cryptococcosis, such as culture and histopathology, are challenging because they require complex laboratory infrastructure, laboratory technicians with training in mycology, and long turnaround times for results (days to weeks).<sup>8-10</sup>

In the Latin America regions, there was an estimation of 1.9 million of PLHIV, and about 132,000 PLHIV were estimated in the Central America Region (CAR), as well as the highest estimated number of new HIV infections each year as reported by UNAIDS gap report.<sup>11</sup> High mortality is seen among persons who develop progressive disseminated histoplasmosis (PDH), and data from a cohort of HIV/AIDS patients with suspected histoplasmosis in Guatemala suggest that mortality from patients with maybe as high as 44% before 2009.<sup>12</sup> Similarly, over 90% of the meningitis cases caused by *Cryptococcus neoformans*, known as cryptococcal meningitis, occur in PLHIV, a condition that represents the main risk factor for development of this mycosis. Cryptococcal meningitis is the most important clinical presentation in these patients and is associated with high mortality rates, from 9% to 70% depending of the world's region.<sup>13</sup>

Rapid diagnostics assays (RDA) directly impact the quality of patient care.<sup>3,7,10,12,14,15,16,17</sup> For opportunistic fungal diseases, RDAs have been shown to impact several aspects of patient care such as increasing the number of histoplasmosis cases detected (up to 3 times compared with conventional assays) and reducing mortality of patients with histoplasmosis and cryptococcosis by 50% compared with conventional assays.<sup>3,7,10,12,15,16,17,18,19</sup> Several RDAs for the diagnosis of histoplasmosis and cryptococcosis are available commercially. They were developed for the detection of circulating fungal antigens (Ag) and are available as enzyme immunoassays (EIA) and in lateral flow assay (LFA) formats. Current World Health Organization (WHO) guidelines for the diagnosis and treatment of cryptococcosis and histoplasmosis in PLHIV recommend Ag detection assays for rapid and accurate diagnosis of both diseases.<sup>20,21</sup> Additionally, both assays have been validated in various laboratories in Latin America, showing high analytical performance (sensitivity and specificity higher than 95%).<sup>22-24</sup>

This report presents the experience of implementation of RDAs for detection of histoplasmosis and cryptococcosis in PLHIV in three Panama, Honduras and Nicaragua.

## 2 | MATERIAL AND METHODS

### 2.1 | Patient population

In this study, hospitalised adult patients (older than 18 years) with advanced HIV disease were enrolled. A diagnostic workup for histoplasmosis and cryptococcosis was performed in hospitalised patients

with advanced HIV disease, defined as a CD4 cell count less than 200 cells/mm<sup>3</sup> or a WHO clinical stage 3 or 4 event at presentation for care.<sup>25</sup> Selection of antigen test was done by clinical suspicion for histoplasmosis or cryptococcosis. Clinical suspicion for histoplasmosis was defined as at least three of the following signs or symptoms: fever, pancytopenia, weight loss, lesions of skin or mucosa, pulmonary lesions on radiography or clinical suspicion of tuberculosis (TB). Clinical suspicion of cryptococcosis was defined as one of the following signs or symptoms: headache, loss of vision, stiff neck or neurological deficit.

The study was carried out in four hospitals in three Central American countries: Hospital A was located in Panama City, Panama, and patients were enrolled between August 2016 to June 2019; Hospital B was located in Tegucigalpa, Honduras and enrolment took place from April 2015 to July 2019; Hospital C was located in San Pedro Sula, Honduras, and patients were enrolled from May 2017 to June 2019; and Hospital D was located in Managua, Nicaragua, and patients were enrolled from February 2016 to September 2018. *Histoplasma* Ag testing was performed between April 2016 to September 2018,<sup>29</sup> in Hospitals A, B and D; and *Cryptococcus* Ag testing was performed from April 2015 to September 2019 (54 months) in all hospitals.

### 2.2 | Laboratory assays

Diagnosis of histoplasmosis and cryptococcosis was done according to the recommendation of WHO guidelines for diagnosing and managing both infections on PLHIV.<sup>20,21</sup> Detection of *Histoplasma* urinary antigen was performed using a commercial EIA kit (Clarus *Histoplasma* GM, product reference HGM201. IMMY). *Cryptococcus* Ag was detected in sera and cerebrospinal fluid (CSF), using a commercial LFA kit (CrAg<sup>®</sup> LFA, product reference CR2003. IMMY). RDAs used on this report were previously validated in PLHIV from Colombia and Guatemala.<sup>22,23</sup> None of the study places were using the antigen detection assays described, reason why before testing samples from patients, all personnel from laboratories participating in this study were trained in assay performance as well as other aspects related to assay implementation. Hospital medical staff was also trained on different aspects of the histoplasmosis and cryptococcosis including clinical, epidemiological, diagnostic and treatment characteristics of both diseases. All laboratories involved in this study were national accredited.

### 2.3 | Statistical analysis

Information was summarised by calculation of absolute and relative frequencies for categorical variables, continuous variables were evaluated for normality, and summary measures were done. Variable associations or differences were identified using chi-square tests, or tests of the null hypothesis (Student's t test or Mann-Whitney U test according to data distribution). These analyses were performed at a 95% confidence level using STATA software version 11.

## 2.4 | Ethical considerations

A non-research determination study protocol for data use analysis was developed and approved by the local and international institutions collaborating in this project.

## 3 | RESULTS

A total of 4,453 hospitalised PLHIV with clinical suspicion of histoplasmosis ( $n = 1,343$ ) or cryptococcosis ( $n = 3,110$ ; 2,721 sera and 389 CSF) were tested during the implementation period (Figure 1). For *Cryptococcus* Ag LFA (CrAg LFA), the largest number of samples was tested in the third quarter of 2017 (July to September;  $n = 434$  samples tested); the median number of samples tested by quarter was 141 samples/quarter (Interquartile range [IQR]: 6–299 samples/quarter) (Figure 2). For *Histoplasma* Ag EIA (HisAg EIA), the second quarter of 2018 (April–June) was the period with most samples tested,  $n = 230$ , a median of 165 samples were tested by quarter (IQR: 39–05 samples/quarter).

In Panama (Hospital A), 857 patients were tested using the *Histoplasma* urinary antigen EIA (HisAg EIA), and 1,001 using the *Cryptococcus* Ag LFA (CrAg LFA); in Honduras (Hospitals B and C), 106 patients were tested using the HisAg EIA, and 681 patients were tested using the CrAg LFA; and in Nicaragua (Hospital D), 380 patients were tested using the HisAg EIA, and 1,428 patients using the CrAg LFA (Figure 1). Of 4,453 patients, 3,271 (74%) were males. Patient median age was 34 years (IQR: 27–42 years); no statistically significant differences were observed in patient age grouped by sex ( $p = 0.127$ ). CD4 cell count data were available for 193 of 4,453 (4%) patients tested; median CD4 cell count was 51 cells/ $\mu$ l (IQR: 24–83 cells/ $\mu$ l).

### 3.1 | Positivity of HisAg EIA

Of 1,343 patients tested for urinary *Histoplasma* Ag 269 (20%) were positive. There were 213 (79%) males and 56 (21%) female patients (sex ratio: 4:1), and the median patient age was 33 years

(IQR: 27–38 years old). CD4 cell count data were available in 17 of 269 (6%) histoplasmosis cases with a median CD4 count of 32 cells/ $\mu$ l (IQR: 27–70 cells/ $\mu$ l). By country, in Panama (Hospital A) from 857 patients tested, 201 (23%) were *Histoplasma* Ag positive; in Honduras, in hospital B, from 106 patients tested, 34 (32%) were *Histoplasma* Ag positive; and in Nicaragua (Hospital D), from 380 patients HisAg EIA tested, 34 (9%) had a positive result (Figure 1).

### 3.2 | Positivity of CrAg LFA

Of 3,110 (11%) patients tested for CrAg, 329 (11%) were positive (224 [68%] sera and 105 [32%] CSF). Two hundred forty-one (73%) were males and 88 (27%) were females (sex ratio: 3:1). The median patient age was 36 years old (IQR: 29–43 years old). CD4 cell count data were available in 106 of 329 (32%) cryptococcosis cases, with a median count of 51 cells/ $\mu$ l (IQR: 24–78 cells/ $\mu$ l). In Panama (Hospital A), 47 of 1,001 patients (5%) were *Cryptococcus* Ag positive; in Honduras, 112 of 681 patients (16%) were *Cryptococcus* Ag positive (72 of 290 [25%] in hospital B, and 40 of 391 [10%] in hospital C); and in Nicaragua (Hospital D), 170 of 1,428 patients (12%) were *Cryptococcus* Ag positive (Figure 1).

## 4 | DISCUSSION AND CONCLUSION

This report describes the experience of the implementation of RDAs for the detection of histoplasmosis and cryptococcosis in three Central American countries. In the study, we reported high test positivity, observing that one fifth (20%) patients tested were HisAg positive, and one tenth (10%) were CrAg positive. Honduras presented the highest test positivity, with 32% of those tested positive for HisAg EIA and 16% of those tested positive for CrAg LFA. This study principally entails the description of the implementation of novel laboratory technologies in facilities without laboratory capacity for the diagnosis of histoplasmosis and cryptococcus. It is important to highlight that the transferred assays were previously well validated and personnel on study sited were training and technical support were offered during the study.<sup>22,23</sup>

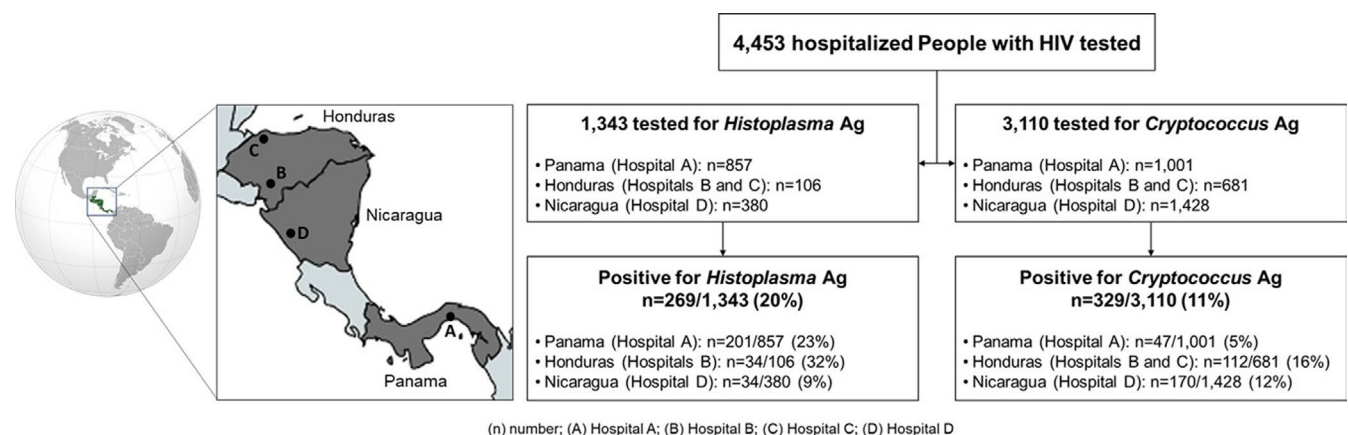
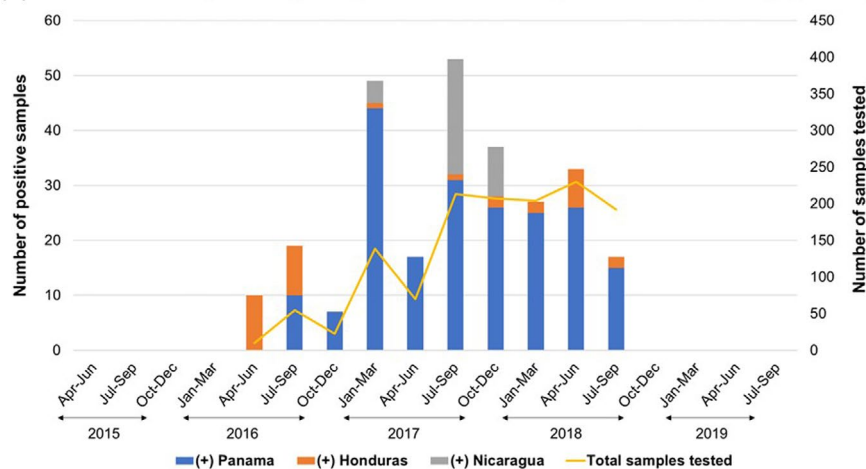


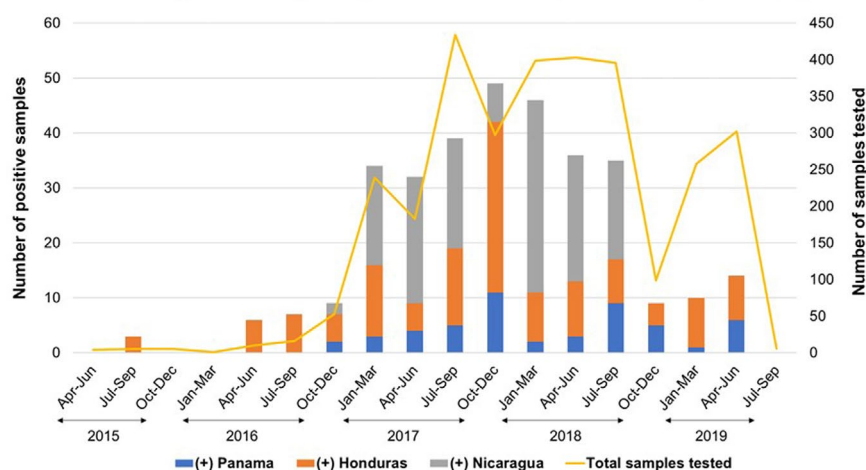
FIGURE 1 Flowchart of patients tested and detected as positive using *Histoplasma* and *Cryptococcus* antigen assays

**FIGURE 2** Timeline: description of total samples tested, and positivity according to antigen assay

**(A) Results of the *Histoplasma* Ag testing: timeline of total samples tested and positivity by country**



**(B) Results of the *Cryptococcus* Ag testing: timeline of total samples tested and positivity by country**



The burden of histoplasmosis and cryptococcosis in PLHIV is unclear in Panama, Nicaragua and Honduras; case series are limited and prior seroprevalence studies for histoplasmosis and case series for cryptococcosis.<sup>4,26</sup> Historically, histoplasmosis has been described as a global travel-related disease, but most of infections reported has been linked with travels to countries in the Americas and the Caribbean region, with multiple histoplasmosis outbreaks reported in people travelling to this region, mostly associated with recreational activities (most commonly cave visits).<sup>27-33</sup> Recently, a modelling study estimated the burden of histoplasmosis in PLHIV based on histoplasmosis seroprevalence studies and regional data about HIV cases in the Latin American region.<sup>4</sup> The authors were able to identify hotspots for histoplasmosis, including the three countries participating in this study, reporting an estimated prevalence of histoplasmosis greater than 30%.<sup>4</sup> A case series from Panama City described 182 cases of histoplasmosis in PLHIV. These cases were diagnosed using conventional diagnostic methods (histopathology and culture) over a period of seven years (from 1997 to 2003).<sup>34</sup> Based on the number of hospitalisations, an 8% prevalence of histoplasmosis was reported; this is much lower than the 23% positivity we found in the Panama hospital in the present study. This discrepancy could be because the use of the HisAg detection assay

increases the number of cases detected up to 3 times compared with conventional assays.<sup>7,18,24</sup>

Data on cryptococcosis in PLHIV from Panama, Nicaragua and Honduras are limited.<sup>35,36</sup> One report from Panama described 28 cases of cryptococcal meningitis identified over a period of five years between 2007 and 2011 (~5 cases/years). However, these patients were diagnosed using India Ink stain and culture.<sup>36</sup> In the present study, in a period of 33 months (2.7 years), we identified 47 patients with positive *Cryptococcus* Ag in Hospital A (from Panama), approximately 12 cases/years. It is known that the sensitivity of the diagnosis of cryptococcal meningitis by culture and direct examination is near 85%. In comparison, antigen detection has a sensitivity near 100%.<sup>37</sup> Additionally, Ag testing can be performed more easily than conventional assays, Ag testing generate results in a couple of hours in comparison with days or weeks need for culture isolation, these factors related to assay performance may have directly impacted case detection.<sup>10,14,38</sup>

In 2017, a diagnostic laboratory in Guatemala tested a total of 1,953 PLHIV for cryptococcosis and histoplasmosis. This laboratory diagnosed opportunistic infections in 16% of PLHIV tested.<sup>7</sup> Histoplasmosis was diagnosed in 120 of 1,850 (7%) of PLHIV tested, and cryptococcosis was diagnosed in 76 of 1,732 (4%) of PLHIV tested.

The prevalence of these infections varies according to the patient's immunological status and are generally observed in PLHIV with advanced disease (CD4 cells <200 cells/mm<sup>3</sup>). The prevalence increased to 11% for histoplasmosis and 9% on cryptococcosis in patients with CD4 cells <200 cells/mm<sup>3</sup>, and in patients with CD4 cell counts <100 cells/mm<sup>3</sup>, prevalence of 15% in histoplasmosis and 11% in cryptococcosis have been reported, frequencies that are like those observed in our study.<sup>7</sup>

There were several limitations to this study, including the inability to access patient's clinical and epidemiological information related to risk factors, treatment, outcomes and long-term impact of RDAs implementation experience. We note that our study does not report prevalence estimates or incidences of these fungal infections, and we limited our description to the report of positive and negative results. All the countries currently follow national and international guidelines on case management for advanced HIV fungal opportunistic infections.<sup>20,21</sup> By obtaining early diagnosis, these guidelines can be implemented correctly.

This study shows the importance of implementing rapid and highly accurate diagnostic assays in resource-limited settings where diagnosis of histoplasmosis and cryptococcosis is limited or lacking, resulting in an impact through early detection for appropriated adequate treatment and case management of both diseases. Since 2017, WHO developed and released guidelines to address advanced HIV disease, including screening, diagnosis and treatment of most common opportunistic infections and co-infections in PLHIV. In addition, WHO in recent years developed specific guidelines for the diagnosis and treatment of histoplasmosis and cryptococcosis among PLHIV, both guidelines recommend the use of Ag testing on these patients.<sup>20,21,25</sup> These assays also present other advantages, high analytical performance and shorter turnaround time for results, in comparison with conventional diagnostics, such as commercial availability of the kits, which facilitates the implementation of these assays in resource-limited settings. Since 2019, the WHO has included these Ag detection assays in the second model list of essential in vitro diagnostics.<sup>39</sup> Further investigation evaluating the impact of these technologies in reduction of morbidity and mortality, economic impact and improvement of patient quality of life are needed.

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## 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 (CDC).

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