

The Diagnostic Laboratory Hub, a new Healthcare system reveals the incidence and Mortality of Tuberculosis, Histoplasmosis and Cryptococcosis of PWH in Guatemala

## Supplementary material

### Microbiological tests

Sputum samples were used for smears (Gram, AFB, Ziehl-Neelsen and Auramine-Rodamine), fungal and mycobacterial cultures and PCR. Microscopy results were available in <24 hours. Mycobacterial and fungal cultures were performed in a BSL-3 facility. For fungal cultures, the sample was inoculated onto Mycosel and Sabouraud agars (BBL, Becton Dickinson). For mycobacterial cultures, sputum samples were digested and decontaminated [2], inoculated onto modified 7H9 Middlebrook liquid media with 800 µL PANTA antibiotic mixture (MGIT® Becton Dickinson) and incubated in the BACTEC®960 system (Becton Dickinson) as well as onto Löwenstein-Jensen medium. Isolator® tubes were centrifuged and the precipitate inoculated onto Mycosel, Sabouraud, Lowenstein-Jensen and BHI agar (BBL, Becton Dickinson). Mycobacteria culture media was incubated at 37°C for 42 days and fungal media at 30°C for 56 days.

Fungi were identified by means of macroscopic and microscopic morphology. Mycobacteria were identified using GenoType Mycobacterium CM and AS hybridization strips (Hain Lifescience GmbH). The results were reported as soon as the identification was complete.

### Immunological tests

*Cryptococcus neoformans/gattii* antigen detection in serum was carried out in every HIV unit by lateral flow assay (CrAg LFA Immy®) according to the manufacturer's

instructions. If positive, a lumbar puncture was recommended to ascertain whether the patient had cryptococcal meningitis. All CSF samples were tested by CrAg LFA and cultured in DLH. The results of CrAg LFA were provided on the same day. As a quality control system of the performance of the test in the HIV units, aliquots of sera from each patient were sent and one in ten were randomly analyzed by CrAg LFA in the DLH. , Indian ink was performed in all CSF samples.

*Histoplasma capsulatum* antigen detection (Ag EIA, Immy®) was performed at the DLH on urine according to the manufacturer instructions. The result was provided in 24 hours.

#### Molecular tests

Sputum samples were collected in 1.5 mL tubes and processed according to Montenegro *et al* [3]. If PCR was not run immediately the DNA was stored at -80°C. PCR for *H. capsulatum* and *M. tuberculosis* on clinical samples were performed according to previously published methods [4,5]. Results were delivered within 72 hours.

The working hours of DHL were from Monday to Friday 7:00-15:00. The electronic laboratory system and e-mail was used to deliver the results and a printed copy then sent via the courier.

#### Treatment

All OIs were treated, if possible. All drugs were supplied by the Health Ministry but when, for any reason, there were not available, the DLH provided it. TB and NTM were treated according to Guatemalan Tuberculosis National Guidelines and the recommendations of ATS/IDSA [6-8]. Cryptococcosis and histoplasmosis were treated

according to the Centers for Disease Control recommendations for the prevention and treatment of opportunistic infections in adults and adolescents with HIV [9].