

Supplementary material

Laboratory procedures:

Each participating healthcare facility (HFC) was requested to send samples of sputum, urine, serum, and 10 ml of blood in an Isolator® tube (Wampole Laboratories, Cranbury, N.J.). Additional samples, such as CSF, were also received depending on the clinical criteria.

Direct microscopy: sputum specimens were analyzed by means of Ziehl–Neelsen (ZN) stain, observed under 100× (oil immersion). Auramine–rhodamine was also used and examined under fluorescent microscope using a blue excitation filter (450 nm).

Cultures: (i) *sputum:* for mycobacteria, specimens were decontaminated with N-acetyl-L-cysteine and 2% NaOH (MycoPrep™, Becton Dickinson, Cockeysville, Md). The supernatant of each specimen was inoculated onto Lowenstein-Jensen medium (LJ) (Becton Dickinson, Cockeysville, Md) and *Mycobacterium* growth indicator tubes (MGIT® Becton Dickinson). For fungal infections, sputum specimens were directly inoculated onto Sabouraud dextrose agar 4% (SDA) (Becton Dickinson, Cockeysville, Md) and Mycosel agar (Becton Dickinson, Cockeysville, Md). (ii) *Cerebrospinal fluid (CSF):* specimens were centrifuged at 3,000 g for 15 min, and the sediment was directly cultured onto SDA, Mycosel, LJ and MIGIT media (iii) *Isolator tube:* blood samples were processed by the lysis-centrifugation system (Isolator; Du Pont Co., Wilmington, Del.). Isolator tubes were centrifuged for 30 minutes at 1800 g. and sediment inoculated onto LJ media for mycobacteria and SDA and Mycosel tubes for fungi. MIGIT and LJ medium were incubated at 37°C, and readings were done daily for a period of 7 days and then once a week for 6 and 8 weeks, respectively. Identification was performed using GenoType *Mycobacterium* CM and AS hybridization strips (Hain Lifescience GmbH). Mycosel and SDA media were incubated at 25°C and the readings were done daily for a period of 7

days and then once a week for 6 weeks. Filamentous fungi growing on the plates were identified based on macroscopic and microscopic features. For yeast identification, VITEK 2 system (bioMérieux, Inc., Hazelwood, MO) was used.

Histoplasma Antigen: Urine samples were evaluated using a commercial enzyme-linked immunosorbent assay (ELISA) for detection of *Histoplasma capsulatum* antigen (IMMY, Norman, OK, USA). This assay was performed according to the manufacturer's instructions.

Cryptococcal antigen (CrAg): Serum and CSF were processed using a lateral flow assay (LFA) test (IMMY, Norman, OK, USA) which was performed according to the manufacturer's instructions. Serum samples were analyzed at the HCFs. To ensure the quality assurance of these results a training program and quality control were developed by the DLH. For those patients with a positive CrAg in serum, a lumbar puncture (LP) was recommended and the CSF obtained was sent to the DLH for CrAg determination and culture.

Polymerase-Chain-Reaction (PCR): Sputum and other specimens were tested with a nested PCR assays targeting the IS6110 insertion for *M. tuberculosis* and a gene for a 100-kDa protein (Hc100 PCR) for *H. capsulatum*. The primers were synthesized by Sigma-Aldrich, Inc., and the DNA extraction and PCR analysis were performed as previously described [8,9].

Molecular and immunology procedures were performed in the biosafety level 2 (BSL-2) and sample processing in BSL-3. Tests were performed daily Monday to Friday, and microscopic and antigen results were reported within 24 hrs, molecular assays within 72 hrs, cultures within 24 hours of turning positive