Histoplasma galactomannan antigen test

Background information

Histoplasma capsulatum, the causative agent of histoplasmosis, is a dimorphic fungus. It lives in soil especially where it is contaminated with bird droppings or bat guano. In the United States, it is typically found in the central and eastern states, especially areas around the Ohio and Mississippi River valleys. Clinically, histoplasmosis may range from an asymptomatic status, to having cough, fever, fatigue, and to a life-threatening disease.

The form that is typically in the environment is the mycelial one that produces tiny spores called microconidia. The latter are able to penetrate deep into the respiratory tree and alveoli. There, under normal body temperature, they change morphology to form yeasts, the pathogenic form. Yeasts can be found in tissues upon biopsy and inside macrophages once phagocytosed, however, they can survive the latter environment, establishing chronic infection in certain individuals. Histoplasmosis may be acute or chronic and localized or disseminated. It is not quite clear what factors set the stage for its increased pathogenicity or chronicity in certain hosts but deficiencies in Th17 pathway and autoantibodies to GM-CSF or IFN-γ are implicated in its pathogenicity especially in the disseminated form where it goes beyond the lungs. There is currently no vaccine but antifungal agents may be used for treatment as indicated. Treatment of disseminated disease especially where it involves CNS is typically prolonged.

Laboratory Diagnosis

Several diagnostic modalities can be used including microscopy (using tissues or body fluids), fungal culture, PCR (where available), serology (to look for antibodies), and antigen testing. Since not all methods have a very high sensitivity and some may require invasive collection methods, it is always best to consider several test modalities to increase overall diagnostic accuracy. Additionally, it is noteworthy that histoplasmosis may clinically overlap with some other infectious diseases (*e.g.* TB, Blastomycosis, *etc*.) or non-infectious ones (such as cancer or autoimmune diseases); so for these reasons, taking clinical, radiological, and epidemiological information into account in addition to lab results are needed for the final diagnosis.

Similar to other fungi, *Histoplasma* has a cell wall composed of many constituents, the important one from diagnostic perspective is galactomannan (GM). This GM is slightly different than that of *Aspergillus spp.* that is commonly used as an aid in diagnosing invasive aspergillosis. As *Histoplasma* divides in the body, its GM is shed in tissues and biological fluids and it eventually gets concentrated in urine. It is for this reason that testing urine is, on average, offers highest sensitivity among other specimen types.

Limitations of the assay

GM is usually phagocytosed by neutrophils, monocytes, and macrophages especially in peripheral blood where neutrophils are abundant. This is one reason why serum is inferior to urine in terms of test sensitivity. For the same reason, sensitivity of *Histoplasma* antigen test is on average lower in individuals who are non-neutropenic, a notion that also applies to *Aspergillus* GM test. *Histoplasma* also shed another major cell wall component, beta-D-glucan (BDG; detected by Fungitell® assay). That being said, a host of other fungi such as *Aspergillus* and *Candida spp*. also shed BDG, making the latter much less specific than *Histoplasma* GM for diagnosing histoplasmosis. The *Histoplasma* GM test is more sensitive in disseminated disease and/or in the immunocompromised setting compared with the localized disease and/or the immunocompetent setting. Antigenuria can be detected in 75% of acute pulmonary infections within the first few weeks compared with less severe disease or chronic cavitary pulmonary histoplasmosis. The test may be undetectable in *Histoplasma* granulomatous mediastinitis and mediastinal fibrosis. *Histoplasma* antibody testing may be more sensitive than antigen testing in the immunocompetent setting. A negative urine antigen test result does not exclude infection. Low positive results may at times be due to cross-reactivity with *Blastomyces*, *Talaromyces marneffei*, *Paracoccidioides*, and some *Candida* spp. The concentrations are only reported for informational purposes. This means where pre-test probability is not high,

a low positive result should be viewed with caution but localized cases such as CNS histoplasmosis may also show low positive urine antigen test results. It is for this reason that one should not use this test for monitoring response to treatment. The current test is only FDA-approved for urine specimen. Other specimens cannot be tested. Should CNS histoplasmosis be clinically suspected but urinary antigen test is negative, one may consider sending CSF specimen out to a lab that has validated CSF for this test. All in all, Clinical, epidemiological, radiological, mycological, serological, and cyto-/histopathological correlation is highly advised.

The performance of this test is unknown when specimens including the following substances are tested: foods which produce color in urine, vaginal cream, caffeine, ascorbic acid, itraconazole, amphotericin B, acetaminophen, or acetylsalicylic acid. Results between different *Histoplasma* assays cannot be compared.

Methodology

The current test is FDA-approved to diagnose active infection with *Histoplasma* under the commercial name *clarus Histoplasma* GM enzyme immunoassay (IMMY). It will be offered at the Immunopathology laboratory at the main campus. This a sandwich ELISA (aka EIA) using rabbit monoclonal antibodies against *Histoplasma* GM. The capture antibody is affixed onto microplates and the detection antibody is conjugated to HRP. After adding the substrate, the color change is read spectrophotometrically. A standard curve using year-phase extract of *Histoplasma* GM is used for interpolation of raw data to actual concentration in nanogram per milliliter (ng/mL).

References

https://www.cdc.gov/fungal/diseases/histoplasmosis/index.html

https://academic.oup.com/cid/article/45/7/807/541502

https://journals.asm.org/doi/10.1128/CMR.00027-06

Test Overview

Test Name	*Histoplasma* galactomannan antigen, urine
Ordering Mnemonic	UHISTO
Methodology	Enzyme-linked immunosorbent assay (ELISA) (aka EIA)
Specimen Requirements	Sterile container: 1.0 mL; Minimum 0.5 mL
Specimen Stability	Up to 48 hours (Ambient), 14 days (Refrigerated), 60 days (frozen ≤ –20°C)
Clinical Information	As an aid in diagnosing active/current *Histoplasma* infection
Reference Range	Negative; <0.2 ng/mL
CPT Code	87385
Days Performed	Tuesday and Friday

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