Gotham Biotech’s Histoplasma Urinary Antigen EIA Kit Now Available for Purchase

Our Histoplasma Urinary Antigen EIA is an enzyme immunoassay intended to qualitatively detect Histoplasma capsulatum galactomannan antigen in human urine.

Gotham Biotech advancing medical diagnostics by designing rapid non-invasive assays

PORTLAND, ME, UNITED STATES, September 8, 2023 /EINPresswire.com/ -- The Gotham Biotech Histoplasma Urinary Antigen EIA is an enzyme immunoassay (EIA) intended to qualitatively detect the presence of Histoplasma capsulatum galactomannan antigen in human urine specimens. This kit, when used in conjunction with other diagnostic measures, can be used as an aid in the diagnosis of histoplasmosis.

INTRODUCTION

Histoplasmosis is a systemic disease caused by the thermally dimorphic fungus Histoplasma capsulatum . H. capsulatum is distributed worldwide and endemic to the Ohio and Mississippi River Valleys in the United States, where the CDC estimates 60% to 90% of people who live in the region have been exposed to the fungus at some point during their lifetime, and to certain regions of Central and South America.

Histoplasmosis is most common among patients positive for HIV or otherwise have a compromised immune system. It is especially a problem in regions of the world where antiretroviral therapy (ART) is not widely available, as ART prevents HIV- infected people from reaching the stage where they are especially vulnerable to histoplasmosis and other opportunistic infections. In Latin America, histoplasmosis is one of the most common opportunistic infections among people living with HIV. There is a 30% mortality rate among HIV/AIDS patients also diagnosed with Histoplasmosis.

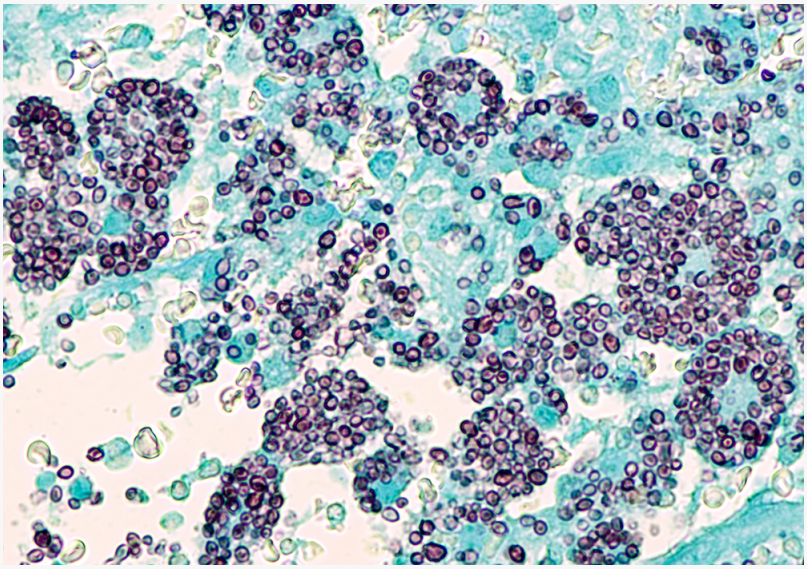
H. capsulatum is typically transmitted by the inhalation of microconidia by the host, deposition in the alveoli, and rapid conversion to a parasitic yeast form in host tissues. Infection is typically

asymptomatic. Among immunocompetent hosts in endemic areas, 95-99% of the primary infections are not recognized or detected.

Because the symptoms of histoplasmosis have significant clinical overlap with other diseases, the definitive diagnosis of histoplasmosis requires either isolation of H. capsulatum from a clinical specimen or direct visualization of the yeast form in clinical specimens. These procedures may require invasive measures to obtain tissues and cultures may take up to six weeks to reveal fungal growth. In contrast, enzyme immunoassays (EIAs) detect H. capsulatum polysaccharide antigen in bodily fluids including urine and blood. Such tests provide rapid results and reasonable specificity and may be used to supplement culture and microscopic examination to diagnose histoplasmosis.

PRINCIPLE OF THE ASSAY

The Gotham Biotech Histoplasma Urinary Antigen EIA incorporates the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique pair of monoclonal antibodies that are specific to distinct epitopes of intact galactomannan molecule antigen. Capture rabbit monoclonal anti-galactomannan antibody is immobilized to the solid phase (microtiter wells) and a detection rabbit monoclonal anti-galactomannan antibody is conjugated with HRP (horseradish peroxidase) enzyme, that generates blue color by oxidizing TMB (3,3',5,5'-Tetramethylbenzidine). The test sample is allowed to react simultaneously with the two antibodies, resulting in the



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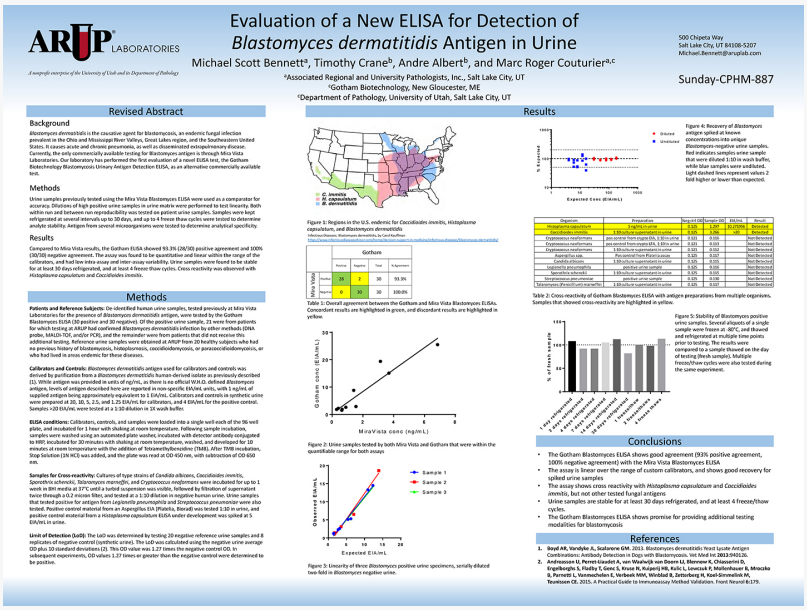

Evaluation of a New ELISA for Detection of *Blastomyces dermatitidis* Antigen in Urine

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Revised Abstract

Background

Blastomyces dermatitidis is the causative agent for blastomycosis, an endemic fungal infection prevalent in the Ohio and Mississippi River Valleys, Great Lakes region, and the Southeastern United States. It causes acute and chronic pneumonia, as well as disseminated extrapulmonary disease. Currently, the only commercially available testing for Blastomyces antigen is through Mira Vista Laboratories. Our laboratory has performed the first evaluation of a novel ELISA test, the Gotham Biotechnology Blastomycosis Urinary Antigen Detection ELISA, as an alternative commercially available test.

Methods

Urine samples previously tested using the Mira Vista Blastomyces ELISA were used as a comparator for accuracy. Dilutions of high positive urine samples in urine matrix were performed to test linearity. Both within-run and between-run reproducibility was tested on patient urine samples. Samples were kept refrigerated at several intervals up to 30 days, and up to 4 freeze-thaw cycles were tested to determine analyte stability. Antigen from several microorganisms were tested to determine analytical specificity.

Results

Compared to Mira Vista results, the Gotham ELISA showed 93.3% (28/30) positive agreement and 100% (30/30) negative agreement. The assay was found to be quantitative and linear within the range of the calibrators, and had low intra-assay and inter-assay variability. Urine samples were found to be stable for at least 30 days refrigerated, and at least 4 freeze thaw cycles. Cross-reactivity was observed with *Histoplasma capsulatum* and *Coccidioides immitis*.

Methods

Patients and Reference Subjects: De-identified human urine samples, tested previously at Mira Vista Laboratories for the presence of *Blastomyces dermatitidis* antigen, were tested by the Gotham Blastomyces ELISA (30 positive and 30 negative). Of the positive urine sample, 21 were from patients for which testing at ARUP had confirmed *Blastomyces dermatitidis* infection by either methods (DNA probe, Nat-HDP, and/or PCR), and the remainder were from patients that did not receive this additional testing. Reference urine samples were obtained at ARUP from 20 healthy subjects who had no evidence of infection with *Blastomyces*, histoplasmosis, coccidioidomycosis, or paracoccidioidomycosis, or who had lived in areas endemic for these diseases.

Calibrators and Controls: *Blastomyces dermatitidis* antigen used for calibrators and controls was derived by purification from a *Blastomyces dermatitidis* human-derived isolate as previously described (1). While antigen was provided in units of ng/mL, as there is no official W.H.O. defined *Blastomyces* antigen, levels of antigen described here are reported in non-specific EIA/mL units, with 1 ng/mL of supplied antigen being approximately equivalent to 1 EIA/mL. Calibrators and controls in synthetic urine were prepared at 20, 10, 5, 2.5, and 1.25 EIA/mL for calibrators, and 4 EIA/mL for the positive control. Samples >20 EIA/mL were tested at a 1:10 dilution in 1X wash buffer.

ELISA conditions: Calibrators, controls, and samples were loaded into a single well each of the 96 well plate, and incubated for 1 hour with shaking at room temperature. Following sample incubation, samples were washed using an automated plate washer, incubated with detector antibody conjugated to HRP, incubated for 30 minutes with shaking at room temperature, washed, and developed for 10 minutes at room temperature with the addition of Tetramethylbenzidine (TMB). After TMB incubation, Stop Solution (1N HCl) was added, and the plate was read at OD 450 nm, with subtraction of OD 650 nm.

Samples for Cross-reactivity: Cultures of type strains of *Candida albicans*, *Coccidioides immitis*, *Sporothrix schenckii*, *Talaromyces marneffei*, and *Cryptococcus neoformans* were incubated for up to 1 week in BHI media at 37°C until a turbid suspension was visible, followed by filtration of supernatant twice through a 0.2 micron filter, and tested at a 1:10 dilution in negative human urine. Urine samples that tested positive for antigen from *Legionella pneumophila* and *Streptococcus pneumoniae* were also tested. Positive control material from an Aspergillus EIA (Platelia, Bio-rad) was tested 1:10 in urine, and positive control material from a *Histoplasma capsulatum* ELISA under development was spiked at 5 EIA/mL in urine.

Limit of Detection (LoD): The LoD was determined by testing 20 negative reference urine samples and 8 replicates of negative control (synthetic urine). The LoD was calculated using the negative urine average OD plus 20 standard deviations (2). This OD value was 1.27 times the negative control OD. In subsequent experiments, OD values 1.27 times or greater than the negative control were determined to be positive.

Results

Figure 1: Regions in the U.S. endemic for *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*

Table 1: Overall agreement between the Gotham and Mira Vista Blastomyces ELISAs. Concordant results are highlighted in green, and discordant results are highlighted in yellow.

Figure 2: Urine samples tested by both Mira Vista and Gotham that were within the quantifiable range for both assays

Figure 3: Linearity of three *Blastomyces* positive urine specimens, serially diluted two-fold in *Blastomyces* negative urine.

Figure 4: Recovery of *Blastomyces* antigen spiked at known concentrations into unique *Blastomyces*-negative urine samples. Three *Blastomyces* positive samples that were diluted 1:10 in wash buffer, while blue samples were undiluted. Light dashed lines represent values 2 fold higher or lower than expected.

Table 2: Cross-reactivity of Gotham Blastomyces ELISA with antigen preparations from multiple organisms. Samples that showed cross-reactivity are highlighted in yellow.

Figure 5: Stability of Blastomyces positive urine samples. Several aliquots of a single sample were frozen at -80°C, and thawed and refrigerated at multiple time points prior to testing. The results were compared to a sample thawed on the day of testing (fresh sample). Multiple Freeze/Thaw cycles were also tested during the same experiment.

Conclusions

- The Gotham Blastomyces ELISA shows good agreement (93% positive agreement, 100% negative agreement) with the Mira Vista Blastomyces ELISA
- The assay is linear over the range of custom calibrators, and shows good recovery for spiked urine samples
- The assay shows cross reactivity with *Histoplasma capsulatum* and *Coccidioides immitis*, but not other tested fungal antigens
- Urine samples are stable for at least 30 days refrigerated, and at least 4 freeze/thaw cycles.
- The Gotham Blastomyces ELISA shows promise for providing additional testing modalities for blastomycosis

References

1. **Boyd AR, Vandyke A, Scalarone GM.** 2013. Blastomyces dermatitidis Yeast Lysate Antigen Combinations: Antibody Detection in Dogs with Blastomycosis. Vet Med Int **2013:**842126.
2. **Andreasson U, Perret-Liaudet A, van Waalwijk van Doorn LJ, Blennow K, Chiasserini D, Engelborghs S, Fladby T, Genc S, Kruse N, Kulaperi HR, Kulic L, Lewczuk P, Mollenhauer B, Mroczko B, Parnetti L, Vanmechelen E, Verbeek MM, Winblad B, Zetterberg H, Kaeil-Simmelink M, Teunissen CE.** 2015. A Practical Guide to Immunoassay Method Validation. Front Neurol **6:**179.

Gotham Biotech has developed strong collaborative associations with ARUP Laboratories as well as the Mayo Clinic for the purpose of evaluating a new ELISA for Detection of Blastomyces dermatitidis antigen in urine (Blasto Urine Test).

galactomannan molecules being sandwiched between the solid phase and enzyme- linked antibodies.

After a 60-minute incubation at room temperature with orbital shaking, the wells are washed with buffer to remove any unbound material. A solution of TMB is added and incubated for 10 min. A blue color develops, indicating presence of the galactomannan antigen in the test sample. After incubation, the color development is stopped with the addition of 1N HCl. The resulting yellow color is immediately measured spectrophotometrically at 450nm and 630nm. Time to result is approximately 1.5 hours.

CONTACT

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