Full Submission for inclusion of an IVD category to the EDL

Survey response 1

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5

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2018-10-31 12:37:39

Last page
8

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en

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2018-10-30 07:50:41

Date last action
2018-10-31 12:37:39

Identification

Please indicate your response ID (unique identifier given to your screening application)
40.0000000000

Name of organization supporting this application:
Global Action Fund for Fungal Infections

Please enter full applicant’s name
David Denning

4. Typical Characteristics of IVD’s in each format in which tests in the test category are available
Please provide detailed description of test components (reagents/instrumentation (where relevant)), methodology and labelling for each test format available

Immy kit - ELISA
The ALPHA Histoplasma Antigen EIA is an immunoenzymatic, sandwich microplate assay used for the qualitative detection of Histoplasma antigens in urine samples.

The ALPHA Histoplasma Antigen EIA is a test which, when used in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiographic evidence, can be used as an aid in the diagnosis of histoplasmosis.

Reagents:
A. Capture Antibody-Coated Microwells (192) (REF HAGMW1) – A stripwell plate featuring breakaway polystyrene microwells. The wells are coated with rabbit anti-Histoplasma IgG antibodies.
B. Histoplasma Antigen Standard – 100 EIA units (1 mL) (REF HAG100) – Histoplasma antigen from strain 288 mycelia phase culture filtrate diluted in buffered protein solution to 100 EIA units and preserved with sodium azide – used in conjunction with other standards to create a standard curve.
C. Histoplasma Antigen Standard – 30 EIA units (1 mL) (REF HAG030) – Histoplasma antigen from strain 288* mycelia phase culture filtrate diluted in buffered protein solution to 30 EIA units and preserved with sodium azide – used in conjunction with other standards to create a standard curve.
D. Histoplasma Antigen Standard – 10 EIA units (1 mL) (REF HAG010) – Histoplasma antigen from strain 288 mycelia phase culture filtrate diluted in buffered protein solution to 10 EIA units and preserved with sodium azide – used in conjunction with other standards to create a standard curve.
E. Histoplasma Antigen Standard – 2 EIA units (1 mL) (REF HAG002) – Histoplasma antigen from strain 288 mycelia phase culture filtrate diluted in buffered protein solution to 2 EIA units and preserved with sodium azide – used in conjunction with other standards to create a standard curve.
F. Positive Control (1mL) (REF HAGPC1) – Histoplasma antigen from strain 288 mycelia phase culture filtrate in a buffered protein solution containing a preservative.
G. Negative Control (1mL) (REF HAGNC1) – Buffered protein solution containing a preservative.

Procedure
Enough microwells should be used for the number of samples and standards per assay.
1. 100 μL of samples, 60 minutes @ 37°C. Decant.
2. Wash 3 times with deionised or distilled water.
3. 100 μL of Detect Antibody, 60 minutes @ 37°C. Decant.
4. Wash 3 times.
5. 100 μL of Streptavidin HRP, 30 minutes @ 25°C, shaking - decant.
6. Wash 3 times.
7. 100 μL Substrate for 10 minutes @ 22-25°C.
8. 100 μL Stop Solution.
9. Measure OD.

Specimens and kit components should be brought to RT (21-25°C) prior to testing.

Equipment
Spectrophotometer microplate reader capable of reading absorbances at 450nm or 450 and 630nm with software capable of generating a four-parameter curve.

Optimum Diagnostics kit
The OIDx 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.

Reagents and Materials Provided
A. Microtitre wells – each kit contains 96 microtitre wells coated with an anti-galactomannan monoclonal antibody. Ready for use.
B. Concentrated Wash Buffer – three vials with 10X PBS 0.5% Tween20 and preservative. Dilute the contents of each bottle 10X with deionized water prior to use. Diluted (1X) wash buffer can be used up to one week after
C. Positive control – ready for use.
D. Negative control – ready for use.
E. HRP Conjugate – one bottle with purified anti-galactomannan monoclonal antibody conjugated to horseradish peroxidase (HRP) in a stabilizing buffer. Ready for use.
F. Color developer – one bottle with chromogenic substrate solution containing tetramethylbenzidine (TMB) and hydrogen peroxide. Ready for use.
G. Stop solution – one vial with 1N HCl. Ready for use.

Equipment required
Microplate reader with 450 nm capability (630 nm capability is optional).
Microplate shaker capable of 600RPM
Microtiter strip holder
Pipettors and/or pipets that can accurately and precisely deliver 100 and 300 μL volumes
Wash solution trough for multichannel pipette
Deionized water for dilution of Concentrated Wash Buffer.

Reagent Preparation
A. All reagents should be allowed to reach room temperature (15-30°C) before use.
B. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

Assay Procedure – Dual Wavelength
1. Pipette 100 μL of well-mixed Positive Control, Negative Control, and Patient specimens into duplicate wells. Include testing of positive and negative controls in duplicate per testing event.
2. Immediately pipette 100 μL of anti-galactomannan HRP-conjugate into all previously pipetted wells.
3. Seal wells with a microplate adhesive cover.
4. Allow the wells to incubate for 60 ± 5 minutes at 15-30°C under constant agitation by a microplate shaker, at 600 ± 50 RPM.
5. After incubation either decant or aspirate the sample and conjugate mixture from the wells. If decanting, be sure to firmly, but carefully, strike the strips on absorbent toweling.
6. Pipette 300 μL of diluted wash buffer into each well. Gently tap the plate to mix the reagents within the wells for 10 to 15 seconds. Decant or aspirate the wash buffer as done in Step 5.
7. Repeat Step 6 twice for a total of three washes.
8. Pipette 100 μL of Color Developer into each well. Incubate for 10 ± 1 minutes at 15-30°C.
9. Pipette 100 μL of Stop Solution into each well ensuring that the Stop Solution is added to the wells in the same order as the Color Developer.
10. Read the optical densities of the wells at both 450 and 630 nm on a microplate reader immediately or within 5 minutes of adding Stop Solution. Do not read results after 5 minutes from adding the Stop Solution. Subtract the optical density at 630 nm from the optical density at 450 nm to correct for background noise.

Assay Procedure – Single Wavelength
1. Read the optical densities of the empty wells to be used in the assay at 450 nm on a microplate reader (“Empty OD450”).
2. Pipette 100 μL of well-mixed Positive Control, Negative Control, and Patient specimens into duplicate wells. Include testing of positive and negative controls in duplicate per testing event.
3. Immediately pipette 100 μL of anti-galactomannan HRP-conjugate into all previously pipetted wells.
4. Seal wells with a microplate adhesive cover.
5. Allow the wells to incubate for 60 ± 5 minutes at 15-30°C under constant agitation by a microplate shaker, at 600 ± 50 RPM.
6. After incubation either decant or aspirate the sample and conjugate mixture from the wells. If decanting, be sure to firmly, but carefully, strike the strips on absorbent toweling.
7. Pipette 300 μL of diluted wash buffer into each well. Hold the plate with one hand and gently tap the plate with other hand to mix the reagents within the wells for 10 to 15 seconds. Decant or aspirate the wash solution as done in Step 6.
8. Repeat Step 7 twice for a total of three washes.
9. Pipette 100 μL of Color Developer into each well. Incubate for 10 ± 1 minutes at 15-30°C.
10. Pipette 100 μL of Stop Solution into each well. Ensure that the Stop Solution is added to the wells in the same order as the Color Developer.
11. Read the optical densities of the wells at 450 nm on a microplate reader immediately or within 5 minutes of adding Stop Solution. Do not read results after 5 minutes from adding the Stop Solution. Blank against “Empty OD450” (see step 1) to correct for background noise.

Interpretation of Results
Dual Wavelength Procedure – the absorbance of each well at 630 nm should be subtracted from the absorbance of each well at 450 nm to obtain a corrected absorbance for use in the calculation of sample results.
Single Wavelength Procedure – the absorbance of each empty well (“Empty OD450”) should be subtracted from the absorbance of each respective well to obtain a corrected absorbance for use in the calculation of sample results.

Validity of test results – Validity of test results is based on expected values for negative control and positive control. The test is
considered invalid if any control results fall outside of their respective expected ranges.

Diagnostic accuracy: Please provide typical sensitivity, specificity, PPV, NPV) for each test format available. Note: Section 5 below request a summary of studies supporting performance criteria shown in this section.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR+</th>
<th>LR-</th>
<th>Diagnostic accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immy kit</td>
<td>80.9%</td>
<td>98.7%</td>
<td>92.7%</td>
<td>96.2%</td>
<td>62.3</td>
<td>0.19</td>
<td>95.7%</td>
</tr>
<tr>
<td>Optimum Imaging diagnostics</td>
<td>94.7% (100% (culture positives))</td>
<td>69.7%</td>
<td>85.7%</td>
<td>87.3%</td>
<td>3.1</td>
<td>0.08</td>
<td>86.1%</td>
</tr>
</tbody>
</table>

Specimen types: Please provide the range of specimen types that can be used with each format for which the tests are available.

- Urine for both kits.

Facility level: the kind of facility in which each test format is intended to be used. Include all that apply.

<table>
<thead>
<tr>
<th>Facility level</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: primary health care clinic with no laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II: district/hospital laboratory</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>III: regional/provincial laboratory</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>IV: national reference laboratory</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

User Skill level: minimum level of training the operator undergoes to effectively perform each of the test formats.

- highly skilled laboratory trained health care worker

Throughput: number of specimens tested at one time for efficient use of each test format.

- low

Time to result: length of time to report the result for each test format.

<table>
<thead>
<tr>
<th>Test format</th>
<th>Time to result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immy kit</td>
<td>4 hours in total</td>
</tr>
<tr>
<td>Optimum Imaging diagnostics</td>
<td>2 hours in total</td>
</tr>
</tbody>
</table>

Environmental stability (temperature, humidity) and shelf life for each test format.

- Operating: 21-25C
- Storage: 2-8C
- Transport: 2-8C

Disposal risks: risks posed by disposal of IVD components for each test format. Include all that apply.

- Biohazard: No
- Comment: No
- Toxic to humans: No
- Toxic to environment: No
<table>
<thead>
<tr>
<th>Disposal risks: risks posed by disposal of IVD components for each test format. Include all that apply. [Plastic]</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
</tr>
<tr>
<td>Disposal risks: risks posed by disposal of IVD components for each test format. Include all that apply. [Comment]</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Disposal risks: risks posed by disposal of IVD components for each test format. Include all that apply. [Other]</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Disposal risks: risks posed by disposal of IVD components for each test format. Include all that apply. [Other comment]</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Quality assurance: please provide the following details for each test format: Control integrated into IVD (Int QC); quality control sample(s) supplied with IVD (Incl QC); quality control available separately at a cost (Sep QC); [Control integrated into IVD (int QC)]</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Quality assurance: please provide the following details for each test format: Control integrated into IVD (Int QC); quality control sample(s) supplied with IVD (Incl QC); quality control available separately at a cost (Sep QC); [Comment]</td>
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</tr>
<tr>
<td>No</td>
</tr>
</tbody>
</table>

5. Evidence summary
Summary of laboratory evaluation studies covering reliability and reproducibility, analytical accuracy (sensitivity, specificity); and analyses of potentially interfering substances, cross reactivity, stability, sample type. All relevant studies should be reported, detailing the search strategy and eligibility criteria used, or providing a systematic review. Please indicate the organizations responsible for conducting each of the studies and provide links to full reports for each study.


Disseminated histoplasmosis, a disease that often resembles and is mistaken for tuberculosis, is a major cause of death in patients with advanced HIV disease. Histoplasma antigen detection tests are an important addition to the diagnostic arsenal for patients with advanced HIV disease and should be considered for inclusion on the World Health Organization Essential Diagnostics List. Our objective was to systematically review the literature to evaluate the diagnostic accuracy of Histoplasma antigen tests in the context of advanced HIV disease, with a focus on low- and middle-income countries. A systematic review of the published literature extracted data on comparator groups, type of histoplasmosis, HIV status, performance results, patient numbers, whether patients were consecutively enrolled or if the study used biobank samples. PubMed, Scopus, Lilacs and Scielo databases were searched for published articles between 1981 and 2018. There was no language restriction.

Of 1327 screened abstracts we included a total of 16 studies in humans for further analysis. Most studies included used a heterogeneous group of patients, often without HIV or mixing HIV and non HIV patients, with disseminated or non-disseminated forms of histoplasmosis. Six studies did not systematically use mycologically confirmed cases as a gold standard but compared antigen detection tests against another antigen detection test. Patient numbers were generally small (19–65) in individual studies and, in most (7/10), no confidence intervals were given. The post test probability of a positive or negative test were good suggesting that this non-invasive diagnostic tool would be very useful for HIV care givers at the level of reference hospitals or hospitals with the infrastructure to perform ELISA tests. The first results evaluating point of care antigen detection tests using a lateral flow assay were promising with high sensitivity and specificity.

No prozone effect is seen.

The antigen is galactomannan antigen shared by other fungi, including Blastomyces dermatitidis (rare HIV pathogen), Coccidioides immitis (occasional HIV pathogen with or without urinary infection), Aspergillus spp. (a cause of invasive disease in HIV patients, with no data on antigen excretion in the urine published in HIV) and low level positivity with Cryptococcus neoformans (which does cause disseminated disease in AIDS and can be found in urine, especially as a persistent nidus in the prostate gland).

Interfering substances tested include blood, epithelial cells, mucus, urinary casts, beta-Hydroxyl butyric acid, ketones, high concentrations of glucose, moderate concentrations of bilirubin and highly acidic urine.

Multiple freeze/thaw cycles reduce ODs. Sample storage at 4°C for > 2 weeks results in a fall in OD, but only low level positives are anticipated to turn negative in such circumstances.

There are few data available on performance outside the Americas, and th strains of Histoplasma capsulatum in Africa, India and SE Asia are slightly different based on taxonomy studies. Work is ongoing in Jakarta to address this.
Histoplasmosis is caused by the thermally dimorphic fungus Histoplasma capsulatum (worldwide distribution) and Histoplasma duboisii (endemic in Africa), is the most common endemic human mycosis (Kauffman 2009). Histoplasma is transmitted by way of the respiratory tract, but once inhaled into the alveoli; the organism readily spreads throughout the body, causing a wide spectrum of manifestations that range from subclinical infections to progressive disseminated disease, affecting both immunocompetent and immunosuppressed individuals (Wheat 2006). The organism grows slowly in vitro and is a Class 3 pathogen, so detection based on culture is too slow and impossible for many centres in LMICs.

Some patients have skin lesions (10-40%) which can be biopsied and a rapid diagnosis made. A rights stain of blood shows circulating yeast cells in monocytes in ~40% of those with disseminated histoplasmosis in AIDS. Apart from antigen detection only PCR or bone marrow aspiration with GMS or PAS staining is sensitive (>90% sensitive) and fast enough to identify all the patients prior to death.

Progressive disseminated histoplasmosis is an increasingly commonly recognised cause of infection in patients with advanced HIV disease from areas endemic for histoplasmosis (Johnson et al. 1986; A. A. Adenis et al. 2014). It is one of the major AIDS-defining infections and a major killer of HIV-infected patients in South and Central America, where it kills ~5,000 - 10,000 people per year of the estimated 24,000 deaths from AIDS there (Adenis, 2018). On the other hand, in the USA, the incidence of AIDS-associated histoplasmosis has declined significantly in the past few years with ARV therapy (Richer et al. 2016; Kauffman 2009).

In the immunocompromised person, Histoplasma polysaccharide antigen detection tests allow rapid diagnosis of disseminated histoplasmosis in urine, serum, bronchoalveolar lavage (BAL), and cerebrospinal fluid (CSF) samples before positive cultures can be identified (Hage et al. 2011). Antigen concentration is greatest in urine and can be used to monitor response to antifungal therapy and to identify relapsing patients. In AIDS patients with disseminated disease, Histoplasma antigen has been detected in the urine of 95-100% and in the serum of 80% of the patients (Wheat et al. 2016; Scheel et al. 2009; Cáceres et al. 2018).

The availability of a simple, rapid method to detect H. capsulatum infection in LMICs would dramatically decrease the time to diagnosis and treatment and deaths in patients with AIDS-related disseminated histoplasmosis. This has been demonstrated in several series using PCR and antigen.


6. Societal impact information

**Ethical issues:** Please detail any important ethical consideration by the type of test and consequences

Unavailability of the test is a major contributor to AIDS deaths in endemic areas, best demonstrated in central America French Guiana and Brazil.

**Equity and human rights issues:** Please indicate if it reduces inequities or increase equity and accessibility

Deaths from AIDS and histoplasmosis typically occurs in the prime of life 35 years. Reduction in these deaths would be beneficial for the affected individuals, families and communities affected.
7. Budget and resources impact

<table>
<thead>
<tr>
<th>Summary of data on comparative cost and cost-effectiveness, if available</th>
</tr>
</thead>
<tbody>
<tr>
<td>None to our knowledge, but cost per assay</td>
</tr>
</tbody>
</table>

Resources and budget impact on health care systems, including specialized human resources, training, maintenance issues as available to support implementation.

Small impact on budgets overall - but best focussed on areas of moderate and high prevalence, which are unknown in Africa in detail, and also some other part of the world.

8. Environmental impact

Please enter any relevant information
None

9. Proposed (new/adapted) text for the EDL.

Please enter any relevant information
Histoplasma antigen detection in urine is an essential diagnostic for ill and hospitalised patients with low CD4 counts and HIV infection, in areas of endemicity and travellers from those areas.

Additional information and signature

Please provide additional information that you would like to be considered.


Through the electronic signature below, I acknowledge that I have provided appropriate information to support this submission. I acknowledge that WHO reserves the right to format and select the information provided as necessary and agree that the information is publicly disclosed by WHO. [Electronic Signature (type your full name to sign):]

David W. Denning

Through the electronic signature below, I acknowledge that I have provided appropriate information to support this submission. I acknowledge that WHO reserves the right to format and select the information provided as necessary and agree that the information is publicly disclosed by WHO. [Date (yyyy-mm-dd):]

2018-10-31