IMMY

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ENGLISH

CLARUS *HISTOPLASMA* GALACTOMANNAN EIA
REF #: HGM201

INTENDED USE

The clarus *Histoplasma* Galactomannan EIA (REF #: HGM201) is an immunoenzymatic, sandwich microplate assay used for the qualitative detection of *Histoplasma* galactomannan in urine samples. When used with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiographic evidence, this test can be used as an aid in the diagnosis of histoplasmosis.

The clarus *Histoplasma* Galactomannan EIA can be used manually or on automated microplate systems. This test is intended to be performed by trained, laboratory professional users.

SUMMARY AND EXPLANATION OF THE TEST

Histoplasma capsulatum (*H. capsulatum*) is a pathogenic dimorphic fungus found worldwide. It is endemic to the Ohio and Mississippi river valleys in the United States and to certain regions of Central and South Americas.[1] Histoplasmosis is caused by breathing in the fungus. It is typically found in dirt with large amounts of bird or bat droppings. It is one of the most frequent mycoses in the world, with over 100,000 cases of disseminated histoplasmosis occurring in AIDS patients worldwide each year.[2] Signs of infection often resemble flu-like symptoms, including fever, cough, fatigue, chills, headaches, chest pain, and/or body aches. Symptoms can appear anywhere from 3 to 21 days after exposure to the fungus occurs.[3] The detection of antibodies to *H. capsulatum* by immunodiffusion and complement fixation are serological methods often used to offer rapid alternatives to microbiological techniques.[3]

The isolation of *H. capsulatum* by culture from clinical specimens remains the definitive diagnosis of histoplasmosis.[4,3] However, culture often requires a two-to-four-week incubation period before the identification of the fungus is possible.[5] In addition, sensitivity of culture is highly variable, and handling of isolates should be done in BSL3 laboratories. A more rational approach to the diagnosis of histoplasmosis and the follow-up of patients may be the rapid detection of *H. capsulatum* antigen (specifically, galactomannan) in urine.[6] The monoclonal antibodies used for detection in this kit have shown clinical utility in diagnosing histoplasmosis in patients.[5,7]

BIOLOGICAL PRINCIPLES

HGM201 is an immunoenzymatic, sandwich microplate assay which detects *Histoplasma* galactomannan in urine. Galactomannan is a polysaccharide found in the fungal cell wall. Monoclonal anti-*Histoplasma* IgG antibodies bound to microwell plates are used as capture antibodies. Horseradish peroxidase (HRP) conjugated anti-*Histoplasma* monoclonal IgG antibodies are used as detection reagents. Urine specimens are run untreated. The samples are added to the microwells coated with the capture antibodies and incubated.

If the patient specimen contains *Histoplasma* galactomannan, those antigens will become bound to the capture antibodies on the microwells. After incubation, the microwells are washed to remove unbound patient material. HRP detection antibodies are added to the microwells. After a second incubation, the microwells are washed to remove any unbound HRP detection antibodies. If antigen is present in the patient sample, a blue color develops with the addition of 3,3',5,5' tetramethylbenzidine (TMB). The reaction is stopped by the addition of Stop Solution, where a yellow color develops. The optical density (absorbance) is checked with a microplate reader at 450 nm and a reference wavelength of 620/630 nm.

EXPLANATION OF THE TWO PROCEDURES

HGM201 has two methods to obtain results using urine samples; the Standard Curve Procedure (four parameter curve fit or linear fit) and the Calibrator Cut-Off Procedure. The user should determine which procedure to use before starting the assay.

The Standard Curve procedure allows for better sensitivity from patient specimen. It uses 7 user-created standards, a positive control, a negative control, and a blank. Results of the procedure are in ng/mL.

The Calibrator Cut-Off Procedure uses fewer microwells by using one user-created calibrator, a positive control, a negative control, and a blank. However, it has reduced sensitivity. Results of the procedure are in EIA units.[8]

REAGENTS PROVIDED

REAGENT	REF#	QTY	DESCRIPTION	Label Symbol	Hazard Symbol
Microwell Plate	HGMMW2	96 ea	A stripwell plate featuring breakaway polystyrene microwells coated with anti-Histo monoclonal Ab	1	N/A
20X Wash Buffer	EIAWB1	50 mL	Concentrated wash buffer containing a preservative.	2	N/A
Standard	HGM100	3 mL	*Histoplasma* galactomannan from culture filtrate diluted in buffered protein solution to 100 ng/mL containing a preservative.	3	
Positive Control	HGMPC2	1 mL	*Histoplasma* galactomannan in a buffered protein solution containing a preservative.	+	
Negative Control	HGMNC2	1 mL	Buffered protein solution containing a preservative.	-	N/A
Conjugate	HGMDA2	10 mL	HRP-conjugated anti-*Histoplasma* IgG monoclonal antibody in a buffered solution containing a preservative.	4	N/A
TMB Substrate	EIATUS	10 mL	Buffered solution containing tetramethylbenzidine (TMB).	5	N/A
Stop Solution	EIASS2	10 mL	Methanesulfonic acid.	6	

REAGENT STORAGE AND STABILITY

- The entire HGM201 test kit should be stored at 2-8 °C until the expiration dates listed on the labels. All reagents should be returned to 2-8 °C promptly after use.
- Unused microwells (1) should be placed back into the resealable Mylar bag and sealed immediately after opening and stored at 2-8 °C. Care should be taken to ensure the desiccant pouch remains in the bag with unused microwells.
- Avoid extended exposure of TMB Substrate (5) to light.
- After preparation, Wash Buffer can be used for 30 days if stored at 2-8°C when not in use. Always check for obvious signs of contamination on each new day of testing.

REAGENT PREPARATIONS

The entire kit, including the microwell plate, should be at 20-25 °C before and during use.
Prepare a 1X solution of Wash Buffer by mixing 19-parts distilled or deionized water with 1-part 20X Wash Buffer (2).

**Refer to the HGM201 SDS for more information on hazards and warnings.*

MATERIALS REQUIRED BUT NOT PROVIDED

A. Pipettors capable of delivering ranges from 100-500 µL and disposable tips

B. Distilled or deionized water

C. Microplate reader capable of reading absorbances at 450 and 620/630 nm with software capable of generating a four-parameter curve fit or linear fit.
D. EIA plate washer or multi-channel pipettor for washing
E. Timer
F. Vortex Mixer
G. Incubator set to 37 °C (±1°C)
H. Graduated cylinder for diluting wash buffer
I. Biohazard waste receptacle

REAGENT PRECAUTIONS

A. Specific standardization is necessary to produce our high-quality reagents and materials.
B. The user assumes full responsibility for any modification to the procedures published herein.
C. Do not use kit or any kit reagents after the stated expiration date.
D. At the time of each use, kit components should be visually inspected for obvious signs of microbial contamination, leakage, or significant physical damage to the microwells. Discard if these conditions are found.
E. IMMY cannot guarantee the performance of its products when used with materials purchased from other manufacturers. Do not interchange reagents from different kit lot numbers or other manufacturers. The use of other products with this test has not been evaluated and may result in erroneous results.
F. Avoid contact with Stop Solution (methanesulfonic acid). If exposed to skin or eyes, immediately flush with copious amounts of water.
G. The following reagents are labeled and have the associated hazards:

For users inside the European Union, the following labeling and hazards apply:
Stop Solution (REF #: EIASS2)

DANGER

H314	Causes severe skin burns and eye damage.
P260	Do not breathe mist/vapor/spray.
P264	Wash hands, forearms, and face thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P303 + P361 + P353	IF ON SKIN (or hair), take off immediately all contaminated clothing. Rinse skin with water.
P305 + P351 + P338	IF IN EYES, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor.
P501	Dispose of contents/container in accordance with local regulations.

Standard (REF #: HGM100) and Positive Control (REF #: HGMPC2)

WARNING

H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.
P261	Avoid breathing mist/vapor/spray.

P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P302 + P352	IF ON SKIN, wash with plenty of water.
P333 + P313	If skin irritation or rash occurs, get medical advice/attention.
P362 + P364	Take of contaminated clothing and wash it before reuse.
P501	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national, and/or international regulation.

Negative Control (REF #: HGMNC2)

H412	Harmful to aquatic life with long lasting effects.
P273	Avoid release to the environment.
P501	Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national, and/or international regulation.

For users outside the European Union, the following labeling and hazards apply:

Stop Solution (REF #: EIASS2)

DANGER

H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
P260	Do not breathe mist/vapor/spray.
P264	Wash hands, forearms, and face thoroughly after handling.
P280	Wear protective gloves, protective clothing, chemical goggles, and face protection.
P301 + P330 + P331	If swallowed, rinse mouth. DO NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair), take off immediately all contaminated clothing. Rinse skin with water or shower.
P304 + P340	IF INHALED, remove person to fresh air and keep comfortable for breathing.
P305 + P351 + P338	IF IN EYES, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor.
P363	Wash contaminated clothing before reuse.
P405	Store locked up.
P501	Dispose of contents/container in accordance with local regulations.

WARNINGS AND PRECAUTIONS FOR USERS

A. For In Vitro Diagnostic Use Only.
B. Avoid splashing when dispensing or aspirating reagents from the microwells as this causes errors.
C. Inadequate washing and inadequate incubation can cause excessive background reactivity in any EIA protocol.
D. Use only protocols described in this package insert. Incubation times or temperatures other than those specified may give errors.
E. Maintain proper pipetting techniques and pattern throughout procedure to ensure optimal and reproducible results.
F. Prepare Wash Buffer by *measuring* one part concentrate to 19 parts deionized water and mixing well.

SPECIMEN COLLECTION AND PREPARATION

Using established techniques by qualified personnel, collect samples aseptically. When handling patient specimens, adequate measures should be taken to prevent exposure to potential etiologic agents. This assay has not been validated on specimens other than urine.

Specimens in transit between labs should be maintained at 2-8 °C. If a delay in specimen processing occurs, storage at 2-8 °C for 72 hours or -16 to -24 °C for up to 2 weeks is permissible. However, a very low-positive specimen could become negative after storage.

Prior to testing, specimens should be brought to 20-25 °C.

TEST PROCEDURE

Step 1	Aliquot enough reagents necessary for tests being run that day, then return the remaining reagents to cold storage *(NOTE: When aliquoting TMB Substrate (5), protect the reagent from light.)*
Step 2	Bring all kit components to 20-25 °C
Step 3	Prepare Standards for either the Standard Curve Procedure (See Table A) or the 12.5 ng/mL Calibrator Cut-Off for the Calibrator Cut-Off Procedure (See Table B) *(NOTE: Be sure to use a new pipette tip for every vial and vortex before performing each serial dilution.)*
Step 4	Snap off a sufficient number of Capture Antibody-Coated Microwells (1) for patient samples, standards, and controls and insert them into the microwell holder, recording the position of each sample, standard, and control. *(NOTE: Place remaining microwells back into bag with desiccant and store at 2-8 °C.)*
Step 5	Add 100 µL of the following to separate microwells: • 1X Wash Buffer (2 diluted to 1X), which serves as the blank for the assay • Positive Control (+) • Negative Control (-) • Standards or Calibrator prepared in Step 3 • Patient samples
Step 6	Mix by gently shaking 1-5 seconds on the countertop or by gently tapping the microwell holder
Step 7	Incubate plate at 37 ± 1 °C for 60 minutes ± 5 minutes
Step 8	Using a pipettor, aspirate the contents from the microwells and discard into a biohazard receptacle changing tips between microwells
Step 9	Wash all microwells with 1X Wash Buffer (2 diluted to 1X), using an EIA plate washer or multichannel pipettor and remove the plate contents after filling, following one of these options: 1. Wash the wells with 370µL of 1X Wash Buffer for a total of 3 washes. 2. Wash the wells with 300µL of 1X Wash Buffer for a total of 5 washes.
Step 10	After the final wash, strike the plate on a clean stack of paper towels or other clean, absorbent material hard enough to remove as much remaining 1X Wash Buffer (2) as possible
Step 11	Add 100 µL of Conjugate (4) to each microwell and repeat Step 6 after all microwells are filled with 4
Step 12	Incubate plate at 37 ± 1 °C for 45 minutes ± 5 minutes
Step 13	Repeat Steps 8-10
Step 14	Add 100 µL of TMB Substrate (5) to each microwell. Start a timer for 30 minutes when 5 is added to the first microwell. Repeat Step 6 after all microwells are filled with 5
Step 15	Incubate at 37 ± 1 °C for the remainder of the 30 minute (± 1 minute) timer set in Step 14
Step 16	Add 100 µL of Stop Solution (6) to each microwell in the same order as Step 14
Step 17	Read and record results *(NOTE: Reading should take place within 15 minutes)*

TABLE A: STANDARD CURVE REAGENTS

Tube	Standards (ng/mL)	Volume of Standard	Volume of 1X [2]
A	25	250 µL [3]	750 µL
B	12.5	500 µL Tube A	500 µL
C	6.25	500 µL Tube B	500 µL
D	3.1	500 µL Tube C	500 µL
E	1.6	500 µL Tube D	500 µL
F	0.8	500 µL Tube E	500 µL
G	0.4	500 µL Tube F	500 µL

TABLE B: CALIBRATOR CUT-OFF REAGENTS

Tube	Calibrator (ng/mL)	Volume of Standard	Volume of 1X [2]
A	25	250 µL [3]	750 µL
B	12.5	500 µL Tube A	500 µL

READING THE TEST

A. Mix by gently tapping the side of the plate or shaking on the countertop for 1-5 seconds.
B. Carefully wipe the undersides of the wells with a clean, lint-free tissue and measure the absorbance of each microwell as outlined on the following
page.
1. A dual wavelength reader is required, with absorbances read at 450 nm and 620/630 nm. Blank on the 1X Wash Buffer (1X [2]). This assay has not been validated with a single wavelength reader.
C. Discard used assay materials as hazardous waste and retain microwell holder.
D. Disinfect the microwell holder with a disinfectant such as:
1. A solution of 10% bleach
2. 70% ethanol
3. 1% Lysol brand I.C.™

Note: If using automation to run the assay, please contact the equipment manufacturer for further instructions.

QUALITY CONTROL & RESULTS

A. The Quality Control and results for each type of run **cannot** be used interchangeably. The standard curve should be run each day of patient testing, do not store the curve for future runs.

B. **STANDARD CURVE PROCEDURE FOUR PARAMETER CURVE FIT**

STANDARD	ACCEPTABLE VALUE
25	1.000 - 2.700 Blanked OD
12.5	0.500 - 1.350 Blanked OD
6.25	0.250 - 0.675 Blanked OD
3.1	0.125 - 0.340 Blanked OD
1.6	0.060 - 0.170 Blanked OD
0.8	0.030 - 0.085 Blanked OD
0.4	0.011 - 0.045 Blanked OD
PC	3.5 - 7.5 ng/mL
NC	< 0.2 ng/mL
1X Wash Buffer (2)	Raw OD < 0.120
R^2	≥ 0.990 Four-Parameter Curve Fit

RESULTS	CONCENTRATION
Negative	<0.20 ng/mL
Positive	≥ 0.20 ng/mL

1. Quality Control:
An assay is considered valid when the Standards, Positive Control (PC), Negative control (NC), and R^2 fall within the acceptable ranges as defined in the tables above.

The Positive Control, Negative Control, and standards must be included with each batch of patient specimens to provide quality assurance of the reagents. The Positive and Negative controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator of standards precision and only ensures reagent functionality.

If the concentration of the Positive Control and/or Negative Control is not within these parameters, patient test results should be considered invalid and the assay should be repeated.

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

2. Results:
Calculate the concentration (ng/mL) by the following:
i. Calculate the average value for each of the standard curve points.
ii. Plot the standard curve values using the following table:

ng/mL (X-Axis)	ABSORBANCE (Y-AXIS)
25	Average of wells (25 ng/mL)
12.5	Average of wells (12.5 ng/mL)
6.25	Average of wells (6.25 ng/mL)
3.1	Average of wells (3.1 ng/mL)
1.6	Average of wells (1.6 ng/mL)
0.8	Average of wells (0.8 ng/mL)
0.4	Average of wells (0.4 ng/mL)

iii. **Using the spectrophotometer software**, calculate a 4-parameter curve fit:

$$Concentration = C\left[\frac{(A-D)}{(Blanked\ OD-D)}-1\right]^{(1/B)}$$

iv. The patient concentrations are calculated by substituting the absorbance values (blanked OD). See below.

EXAMPLE RESULTS AND CALCULATIONS*:

CURVE FIT PARAMETERS	CALCULATED VALUES
A	-0.019
B	0.964
C	496
D	47.3

Using the standard curve equation shown above, for a sample that gives a blanked OD absorbance of 0.695, the concentration would be calculated as follows:

$$Concentration = C\left[\frac{(A-D)}{(Blanked\ OD-D)}-1\right]^{(1/B)}$$

$$Concentration = 496\left[\frac{(-0.019-47.3)}{(0.695-47.3)}-1\right]^{(1/0.964)} = 6.501ng/ml$$

**Numbers here are only intended as examples for demonstration purposes, do not use these numbers for your own calculations*

C. **STANDARD CURVE PROCEDURE LINEAR FIT**

STANDARD	ACCEPTABLE VALUE
25	1.000 - 2.700 Blanked OD
12.5	0.500 - 1.350 Blanked OD
6.25	0.250 - 0.675 Blanked OD
3.1	0.125 - 0.340 Blanked OD
1.6	0.060 - 0.170 Blanked OD
0.8	0.030 - 0.085 Blanked OD
0.4	0.011 - 0.045 Blanked OD
PC	3.5 - 7.5 ng/mL
NC	< 0.2 ng/mL
1X Wash Buffer (2)	Raw OD < 0.120
R^2	≥ 0.990 Linear Curve Fit
Y-Intercept	-0.025 - 0.030

RESULTS	CONCENTRATION
Negative	<0.20 ng/mL
Positive	≥ 0.20 ng/mL

1. Quality Control:
An assay is considered valid when the Standards, Positive Control (PC), Negative control (NC), R^2, and Y-Intercept fall within the acceptable ranges as defined in the tables above.

The Positive Control, Negative Control, and standards must be included with each batch of patient specimens to provide quality assurance of the reagents. The Positive and Negative controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator of standards precision and only ensures reagent functionality.

If the concentration of the Positive Control and/or Negative Control is not within these parameters, patient test results should be considered invalid and the assay should be repeated.

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

2. Results:
Calculate the concentration (ng/mL) by the following:
i. Calculate the average value for each of the standard curve points.
ii. Plot the standard curve values using the following table:

ng/mL (X-Axis)	ABSORBANCE (Y-AXIS)
25	Average of wells (25 ng/mL)
12.5	Average of wells (12.5 ng/mL)
6.25	Average of wells (6.25 ng/mL)
3.1	Average of wells (3.1 ng/mL)
1.6	Average of wells (1.6 ng/mL)
0.8	Average of wells (0.8 ng/mL)
0.4	Average of wells (0.4 ng/mL)

iii. Using the spectrophotometer software, or other appropriate means, calculate a linear fit:

$$Y = mx + \mathrm{b}$$

iv. The patient concentrations are calculated by substituting the absorbance values (blanked OD). See below.

EXAMPLE RESULTS AND CALCULATIONS*:

LINEAR FIT PARAMETERS	CALCULATED VALUES
m (Slope)	0.0731
b (Y-Intercept)	0.0232

Using the standard curve equation shown above, for a sample that gives a blanked OD absorbance of 0.695, the concentration would be calculated as follows:

$$0.695 = 0.0731x + 0.0232$$

$$0.695 = 0.0731(concentration) + 0.0232$$

$$concentration = \frac{0.695 - 0.0232}{0.0731}$$

$$9.19\ ng/mL = \frac{0.695 - .0232}{0.0731}$$

**Numbers here are only intended as examples for demonstration purposes, do not use these numbers for your own calculations*

D. **CALIBRATOR CUT-OFF PROCEDURE**

CONTROLS	ACCEPTABLE VALUE
12.5 Std	0.500 - 1.350 Blanked OD
PC	2.5 - 7.0 EIA Units
NC	<1.0 EIA Units
1X Wash Buffer (2)	Raw OD < 0.120

RESULTS	EIA UNITS
Negative	<1.0 EIA Units
Positive	≥ 1.0 EIA Units

1. Quality Control:
An assay is considered valid when the 12.5 ng/mL Calibrator Cut-Off (CC), Positive Control (PC), and Negative Control (NC) fall within the acceptable ranges as defined in the table above.

The Positive Control, Negative Control, and 12.5 ng/mL Calibrator Cut-Off must be included with each batch of patient specimens to provide quality assurance of the reagents. The Positive and Negative controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator of the Calibrator Cut-Off precision and only ensures reagent functionality.

If the EIA units of the Positive Control and/or Negative Control are not within these parameters, patient test results should be considered invalid and the assay should be repeated.

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

2. Results:

Calculate concentration (EIA Units) by the following:

i. Calculate the average value for the 12.5 ng/mL standard.

ii. Using the following equation, calculate sample EIA Units:

$$EIA\ Units = \left[\frac{Sample\ Blanked\ OD}{(12.5\ Std\ Blanked\ OD)}\ X\ 10\right]$$

EXAMPLE:

$$\left[\frac{0.327}{0.812}\ X\ 10\right] = 4.03\ EIA\ Units$$

iii. Using the table above, compare the sample EIA Units to determine a positive or negative result.

PERFORMANCE CHARACTERISTICS

A. STANDARD CURVE PROCEDURE

1. Interference:

HGM201 was evaluated for the potential of interference on the following urine conditions: bloody urine, proteinuria, mucus, casts, epithelial cells in urine, ketonuria, and bilirubin. These samples exhibit no interference in the assay.

2. Cross-Reactivity:
HGM201 was evaluated for cross-reactivity against a panel of patient specimens across a variety of different pathologies. The results of this testing are shown in the table below.

Pathology	Number of Specimens	% Positive
*Paracoccidioides**	1	100% (1/1)
Blastomyces	11	64% (7/11)
Candida	10	10% (1/10)
Coccidioides Ab	9	0% (0/9)
Aspergillus	8	0% (0/8)
Cryptococcus	5	0% (0/5)
Verticillium	1	0% (0/1)
Mucormycosis	1	0% (0/1)
Pneumocystis	3	0% (0/3)
Malassezia	1	0% (0/1)

**Indicates use of ID antigen as no patient specimen available for Performance Characteristics*

3. Method Comparison:
The HGM201 was compared to IMMY's ALPHA *Histoplasma* EIA (HAG102) in-house. A total of 277 urine specimens were tested on both assays according to their package inserts. Analysis of this data was performed to determine percent agreement positive and percent agreement negative. The results of this comparison are shown in the tables below:

		IMMY HAG102	
		Pos.	Neg.
IMMY clarus HGM201 EIA	Pos.	48	35
	Neg.	10	184

	Calculated	Calculated 95% CI
% Positive Agreement	82.76%	70.57%-91.41%
% Negative Agreement	84.02%	78.48%-88.61%

The comparison to HAG102 resulted in 35 "false-positives" on HGM201. After further analysis of those specimens, it was concluded that 27 of those were proven histoplasmosis cases. Out of the 8 "false-positives" that were not proven histoplasmosis cases, 6 were *Blastomyces* sp. culture-confirmed cases, which has shown to be cross-reactive on the assay. The following tables show this information:

		IMMY HAG102	
		Pos.	Neg.
IMMY clarus HGM201 EI	Pos.	48	2
	Neg.	10	184

	Calculated	95% CI
% Positive Agreement	82.76%	70.57%-91.41%
% Negative Agreement	98.92%	96.17%-99.87%

4.Precision:
Precision (Reproducibility) on HGM201 was evaluated at IMMY by running standards and a panel of five specimens on HGM201 according to Clinical and Laboratory Standards Institute (CLSI) EP-17A. The specimen panel was tested once a day for a total of 5 days. Samples tested: Standards (made following package insert), three positive urines (high positive, medium positive, and low positive), and two negative urines.

For precision, the mean, standard deviation, and %CV were calculated for the blanked OD and concentration values. The results are shown in the table below:

Concentration			
	Mean (ng/mL)	SD	%CV
High Positive Urine	26.398	1.179	4.466
Medium Positive Urine	7.156	0.308	4.298
Low Positive Urine	1.262	0.154	12.165
Negative Urine 1	0.000	0.000	0.000
Negative Urine 2	0.000	0.000	0.000
Positive Control	5.557	0.387	6.961
Negative Control	0.000	0.000	0.000

B. CALIBRATOR CUT-OFF PROCEDURE

1. Interference:
HGM201 was evaluated for potential of interference on the following urine conditions: Bloody urine, Proteinuria, Mucus, Casts, Epithelial cells in urine, Ketonuria, and Bilirubin. These samples exhibit no interference in the assay.

2. Cross-Reactivity:
HGM201 was evaluated for cross-reactivity against a panel of patient specimens across a variety of different pathologies. The results of this testing are shown in the table below:

Pathology	Number of Specimens	% Positive
*Paracoccidioides**	1	100% (1/1)
Blastomyces	11	55% (6/11)
Candida	10	0% (0/10)
Coccidioides Ab	9	0% (0/9)
Aspergillus	8	0% (0/8)
Cryptococcus	5	0% (0/5)
Verticillium	1	0% (0/1)
Mucormycosis	1	0% (0/1)
Pneumocystis	3	0% (0/3)
Malassezia	1	0% (0/1)

**Indicates use of ID antigen as no patient specimen available for Performance Characteristics*

3. Method Comparison

HGM201 was compared to HAG102 in-house. A total of 277 urine specimens were tested on both assays according to their package inserts. Analysis of this data was performed to determine percent agreement positive and percent agreement negative. The results of this comparison are shown in the tables below:

		IMMY HAG102	
		Pos.	Neg.
IMMY clarus HGM201 EIA	Pos.	47	31
	Neg.	11	188

	Calculated	95% CI
% Positive Agreement	81.03%	68.59%-90.13%
% Negative Agreement	85.84%	80.51%-90.18%

The comparison to HAG102 resulted in 31 "false-positives" on HGM201. After further analysis of those specimens, it was concluded that 25 of those were proven histoplasmosis cases. Out of the 6 "false-positives" that were not proven histoplasmosis cases, 5 were *Blastomyces* culture-confirmed cases, which has shown to be cross-reactive on the assay. The tables below show this information:

		IMMY HAG102	
		Pos.	Neg.
IMMY clarus HGM201 EIA	Pos.	47	1
	Neg.	11	188

	Calculated	95% CI
% Positive Agreement	81.03%	68.59%-90.13%
% Negative Agreement	99.47%	97.09%-99.99%

4.Precision:

Precision (Reproducibility) on HGM201 was evaluated at IMMY by running standards and a panel of five specimens on HGM201 according to Clinical and Laboratory Standards Institute (CLSI) EP-17A. The specimen panel was tested once a day for a total of 5 days. Samples tested: Standard (made following package insert), three positive urines (high positive, medium positive, and low positive), and two negative urines.

For precision, the mean, standard deviation, and %CV were calculated for the blanked OD and EIA Unit Values. The results are shown in the table below:

Concentration			
	Mean (EIA units)	SD	%CV
High Positive Urine	24.436	0.712	2.9%
Medium Positive Urine	5.164	0.292	5.6%
Low Positive Urine	0.731	0.097	13.2%
Negative Urine 1	-0.235	0.036	-15.3%
Negative Urine 2	-0.227	0.053	-23.3%
Positive Control	3.813	0.322	8.5%
Negative Control	-0.098	0.047	-48.3%

LIMITATIONS

• HGM201 is intended for use with urine specimens. This assay has not been validated on specimens other than urine.

• Only allow the needed volume of reagents required for the number of microwells to be used to warm to room temperature. Performance of reagents is not determined when exposed to temperature fluctuations.

• A negative result does not exclude diagnosis of histoplasmosis.

• HGM201 was found to be cross-reactive with *Paracoccidioides*, *Blastomyces*, and some *Candida* specimens. Positive tests should be confirmed in areas or patient groups where these organisms are endemic or a risk.

• HGM201 is not intended for monitoring therapy.

• Inadequate washing during the test procedure can cause excessive background reactivity.

• Use only protocols described in this package insert. Incubation times or temperatures other than those specified might give inaccurate results.

• The performance of HGM201 has not been established for manual reading and/or visual result determination.

• Testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative result depends on the pretest likelihood of histoplasmosis disease being present. Testing should only be done when clinical evidence suggests the diagnosis of histoplasmosis.

• It is possible for negative patient specimen microwells to be contaminated by positive control/patient specimen microwells if the contents of one microwell spill over into another microwell. This could be due to rough handling of the microplate or poor pipetting technique while adding reagents.

• Even though it was not tested in HGM201, *Talaromyces marneffei* is known to cross-react with *Histoplasma* antibodies.

• The performance of HGM201 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 capsulatum* assays cannot be compared.

HAZARD AND PRECAUTIONARY INFORMATION

Refer to the product Safety Data Sheets (SDS) for Hazards and Precautionary Statements.

TROUBLESHOOTING

PROBLEM	SOLUTION
Variable results across replicates	• Set-up standards, controls, and samples in a separate, clean, non-coated 96-well plate • Use multichannel to pipette from the 96-well plate into the microwells (1)
Suspected contamination of microwells	• Gently tap the plate to mix reagents in microwells to avoid splashing • Take precaution when pipetting to ensure no splashing or carry-over from neighboring microwells occurs • Change tips between microwells

PROBLEM	SOLUTION
Standard curve failing	• Take care when prepping standards: vortex between dilutions, use precise and careful pipetting techniques, and change tips between dilutions
Lower ODs than expected (Reagents too cold)	• Make sure all reagents come to 20-25°C before testing • Store reagents in a temperature-controlled environment (e.g. incubator) when bringing them to 20-25°C. This can be done by placing reagents in a 20-25°C incubator for 30 minutes or a 37°C incubator for 10 minutes.
Edge effects?	• Edge effects have been found to be insignificant
Using samples other than urines?	• This assay is for urine specimens only

SYMBOLS

	Contains sufficient for XX determinations		Catalogue Number/Reference Number
	Consult instructions for use		Batch/Lot Number
	Manufacturer		For In Vitro Diagnostic Use
	Use By/Expiration date		Authorized Representative in the European Community
	Temperature Limitation		Control
	CE mark of conformity		Single Use Only
	Prescription Use Only		

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