clarus

ASPERGILLUS GM

ENZYME IMMUNOASSAY

For the Qualitative Detection of *Aspergillus* Galactomannan – REF AGM101

℞ ONLY

IVD For In Vitro Use Only

2°C 8°C

IMMY

Rev. 1
Rev. Date 2023-01-27

INTENDED USE

The clarus *Aspergillus* Galactomannan EIA (AGM EIA) is a non-automated, immunoenzymatic, sandwich microplate assay used for the qualitative detection of *Aspergillus* galactomannan in serum and bronchoalveolar lavage (BAL) samples from patients at-risk for invasive aspergillosis.

The clarus AGM EIA is a test which can be used as an aid in the diagnosis of aspergillosis when tested in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiographic evidence. It is intended for laboratory professional use.

SUMMARY AND EXPLANATION OF THE TEST

Aspergillus spp. are ubiquitous filamentous fungi found worldwide and can live both indoors and outdoors. Invasive aspergillosis (IA) is caused by breathing in these fungal spores. IA is one of the most significant threats to recipients of hematopoietic stem cell and solid organ transplants. Individuals with suppressed immune systems due to illnesses such as HIV/AIDS infection are also at a high risk[1-3]. Non-traditional risk factors that have been identified more recently for IA include ICU stays and respiratory viral infections[4]. There has been a significant rise in the incidence of IA in the last two decades due to the widespread use of treatments for some of these conditions, such as chemotherapy and immunosuppressive agents[5-6]. It has been reported that *Aspergillus* infections account for up to 41% of infections within all transplant patients and have a staggering mortality rate of up to 92% within this population[2]. IA is difficult to diagnose, thus requiring a multidimensional approach which includes patient characteristics, clinical and radiological findings, and mycological evidence[7-9]. Early detection and treatment of infection are key to reducing the mortality associated with this disease[10-11].

The clarus AGM EIA is a non-automated immunoenzymatic, sandwich microplate assay that detects *Aspergillus* galactomannan in serum and BAL samples. It can be used as an aid in the diagnosis of aspergillosis when tested in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiographic evidence.

BIOLOGICAL PRINCIPLES

The clarus AGM EIA is a non-automated immunoenzymatic, sandwich microplate assay which detects *Aspergillus* galactomannan in serum and BAL. Controls, serum and BAL specimens require heat pretreatment prior to testing. After pretreatment, the supernatant from the specimen and controls is pipetted into microwells coated with anti-*Aspergillus* antibodies. The microwells undergo incubation at 37°C and are then washed before the addition of the conjugate. After a second incubation (37°C) and wash, 3,3',5,5' tetramethylbenzidine (TMB) is added. Microwells undergo a final incubation (37°C) and then Stop Solution is added. The clarus AGM EIA can be run in under 3 hours, reading results within 15 minutes of completing the test.

Monoclonal anti-*Aspergillus* IgG antibodies are bound to microwell plates and used as capture antibodies. Horseradish peroxidase (HRP) conjugated anti-*Aspergillus* monoclonal IgG antibodies are used as detection reagents. Galactomannan is a polysaccharide found in the cell wall. If the patient specimen contains *Aspergillus* galactomannan, those antigens will bind to the capture antibodies on the microwells. Before the addition of the conjugate, the microwells are washed, which removes any unbound antigen from the well. This reduces the risk of false-negative results due to high-dose hook effect. Once the conjugate is added, the capture antibody-antigen complex binds to the HRP-linked detection antibodies in the conjugate solution. The microwells are washed a second time to remove any unbound material. If antigen is present in the patient sample, a blue color develops with the addition of TMB. The reaction is stopped by the addition of Stop Solution, where a yellow color develops. The optical density is found using a microplate reader at 450 nm and a reference wavelength of 620/630 nm. Corrected OD values are calculated and then blanked before calculating EIA Units.

REAGENTS PROVIDED

REAGENT	REF#	QTY	DESCRIPTION	Label Symbol	Hazard Symbol
Microwell Plate	AGMMW1	96 micro-wells	1 96-well plate, coated with anti-*Aspergillus* monoclonal IgG antibodies, packaged into a mylar pouch with a desiccant pouch	1	N/A
20X Wash Buffer	AGMWB2	50 mL	20X EIA wash buffer; contains 0.4% Tween20, 0.2% ProClin	2	N/A
Pretreatment Buffer	AGMSTB	10 mL	Contains 4% EDTA solution, 0.2% ProClin	3	
Calibrator Cutoff	AGMCC1	1.5 mL	1-5 ng/mL *Asp.* galactomannan in a BSA solution; contains < 0.2% ProClin	4	N/A
Positive Control	AGMPC1	1.5 mL	5-15 ng/mL *Asp.* galactomannan is BSA solution; contains < 0.2% ProClin	+	N/A
Negative Control	AGMNC1	1.5 mL	BSA solution contains < 0.2% ProClin	-	N/A
Conjugate	AGMDA1	10 mL	< 0.3 mg/mL HRP-conjugated *Aspergillus* antibodies	5	N/A
Substrate	EIATUS	10 mL	Buffered solution containing tetramethylbenzidine (TMB)	6	N/A
Stop Solution	EIASS2	10 mL	< 5% Methanesulfonic acid	7	

REAGENT STORAGE AND STABILITY

- The entire AGM EIA 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.

- Avoid extended exposure of Substrate (6) to light.
- Unused microwells (1) should be placed back into the resealable Mylar bag, 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.
- 1X Wash Buffer (2 diluted to 1X) can be used for 14 days if stored at 2-30°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 strips from the Microwell Plate (1), should be at 18-25°C before and during use. Break off enough strips to be tested that day and place unused strips back into the resealable bag (see above).
- Prepare a 1X solution of Wash Buffer by mixing 19-parts DI water with 1-part AGMWB2 (2). Use 1X Wash Buffer as the blank.
- AGMCC1 (4), AGMPC1 (+), and AGMNC1 (-) must be treated with AGMSTB (3). The blank is not treated.
- The following reagents are ready to use: AGMSTB (3), AGMDA1 (5), EIATUS (6), and EIASS2 (7).

REAGENT PRECAUTIONS

A. 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**.

B. The user assumes full responsibility for any modification to the procedures published herein. Use only protocols described in this package insert. Incubation times or temperatures other than those specified in the procedure listed below have not been evaluated and may produce inaccurate results.

C. Do not use kit or any kit reagents after the stated expiration date.

D. The Pretreatment Buffer (REF#: AGMSTB) and Stop Solution (REF#: EIASS2), are labeled:

H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338	IF IN EYES, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P317	If eye irritation persists, Get medical advice/attention.
H402	Harmful to aquatic life.
P273	Avoid release to the environment.
P501	Dispose of contents/container in accordance with local regulations.
H290	May be corrosive to metals.
P234	Keep only in original container.
P390	Absorb spillage to prevent material damage.
P406	Store in corrosive resistant container with a resistant inner liner.

HAZARD AND PRECAUTIONARY INFORMATION

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

WARNINGS FOR USERS

A. **For In-Vitro Diagnostics use Only.**
B. Rx Only
C. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and the patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
D. Maintain proper pipetting techniques and pattern throughout procedure to ensure optimal and reproducible results.
E. Avoid splashing when dispensing or aspirating reagents from the microwells as this causes errors.
F. Biological spills should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach, 70% ethanol, or a 0.5% Wescodyne Plus™. Materials used to wipe spills may require biohazardous waste disposal.
G. Inadequate washing can cause excessive background reactivity in any EIA protocol.
H. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.
I. Avoid contact with Stop Solution (methanesulfonic acid). If exposed to skin or eyes, immediately flush with copious amounts of water.
J. Refer to Hazards and Precautionary information sections for hazards associated with specific reagents. Safety Data Sheets are available upon request.

PRECAUTIONS FOR USERS

A. This assay should only be performed by trained, laboratory professional users.
B. **FROZEN SERUM OR BAL SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE FALSE POSITIVE RESULTS DUE TO CONTAMINATION WITH FUNGI AND/OR BACTERIA.**
C. Do not use kit or any kit reagents after the stated expiration date.
D. Use clean, dust-free materials to minimize the possibility of contamination with *Aspergillus* spores from the environment. Because galactomannan is heat-stable, sterilization of material used does not guarantee the absence of contaminating antigen. Pyrogen-free materials are optimal, but standard material can be used with adequate precautions.
E. Do not pour any unused reagent back into the original container.
F. Maintain proper pipetting techniques and pattern throughout procedure to ensure optimal and reproducible results. When pipetting controls and specimens, use individual pipette tips to prevent carryover of samples.

G. Limit exposure of samples and kit components (sera, BAL fluid, buffers, controls) or open containers (plates, tubes, pipette tips) to the air.
H. Do not use any microwells that were stored in an open Mylar bag, as exposure to moisture/humidity may lead to inaccurate results.
I. A dual wavelength reader is required, with absorbances read at 450 nm and 620/630 nm. This assay has not been validated with a single wavelength reader or at any other reference wavelengths.
J. Do not rely on temperature displayed by heating apparatus. Heat block/water bath temperature should be confirmed by a separate, calibrated thermometer to independently assess actual heat temperature. 120°C must be reached inside the heat block and 100°C must be reached inside the water bath.
K. Do not switch back and forth between heat treatment methods. Only one method should be performed based on laboratory capabilities.
L. Take precaution to avoid burn injury if using water bath to conduct heat treatment method.
M. Only pretreat the number of specimens that will fit in a balanced configuration in the centrifuge. Avoid delays in processing during the pretreatment. For optimal reactivity specimens should be centrifuged **immediately**.
N. Results read after the 15-minute reading window are invalid.
O. Results between different *Aspergillus* galactomannan assays cannot be compared.

SPECIMEN COLLECTION

Collect samples aseptically using established techniques by qualified personnel. When handling patient specimens, adequate measures should be taken to prevent exposure to potentially present etiologic agents. The use of specimens other than serum or BAL has not been established. For optimal results, aseptically collected samples should be used.

Specimens in transit between labs should be maintained at 2-8°C. Process and test samples upon arrival. If a delay is encountered in specimen processing, storage for up to 2 weeks at <-20°C or 10 days at 2-8°C is permissible. For longer storage, store specimen at -80°C. Specimens can be subjected to a maximum of 5 freezing / thawing cycles. A very low-positive specimen could become negative after storage. Previously frozen specimens should be thoroughly mixed after thawing prior to testing. Specimens should be brought to room temperature prior to testing (18-25°C).

SPECIMEN PREPARATION

Calibrator, Positive, and Negative Controls must be pretreated prior to testing. The blank is <u>not</u> pretreated prior to testing. All controls (AGMCC1, AGMPC1, AGMNC1, Blank) must be tested on every run. Results from AGMCC1, AGMPC1, AGMNC1, and Blank are used to validate the run (see Quality Control & Expected Results). Treat appropriate controls for each run at the same time as serum/BAL specimen. **Two methods for heat treatment are outlined below. Only one method should be performed based on laboratory capabilities**. Success of the test requires strict compliance with the prescribed temperature and equipment capacity.

Pretreatment of Controls, Serum and BAL:

1. Place 100 µL of Pretreatment Buffer (3) into individual screw cap, heat-resistant microcentrifuge tubes (or other locking tubes).
2. Add 300 µL of AGMCC1, AGMPC1, AGMNC1, fresh serum, or BAL to each pretreatment tube. The blank is not pretreated.
3. Screw the lids on tightly to prevent opening during heating, and vortex the samples.
4. Heat-treat all samples and appropriate controls using **one of the following methods** based on laboratory capabilities*:
   Heat block option: Place tubes in a heat block for 6-8 minutes at 120°C.
   OR
   Water bath option: Place tubes in a water bath for 6-8 minutes at 100°C.
5. Remove tubes from the heat block or the boiling water bath, and **immediately** place into centrifuge.
6. Spin sample for 5 minutes at 10,000-14,000 x g at room temperature (18-25°C).
7. Remove microcentrifuge tubes and test the supernatants following the Test Procedure. If needed, pretreated specimens (supernatant with pellet) and controls can be stored at 2-8°C for up to 24 hours prior to testing.

**See Precautions for Users*

TEST PROCEDURE

Step 1	Aliquot enough reagents necessary for tests being run that day. Return the remaining reagents to cold storage (2-8°C). Bring all aliquoted reagents and the mylar bag containing the Microwell Plate (1) to 18-25°C. *(NOTE: When aliquoting Substrate (6), protect the reagent from light)*
Step 2	Break off enough capture antibody-coated strips from the Microwell Plate (1) for Positive Control (+), Negative Control (-), Blank (2 diluted to 1X), Calibrator Cutoff (4), and patient samples and insert them into the microwell holder. Unused microwells should be placed back into the resealable mylar bag, **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. Use one microwell for the Positive Control (+), one microwell for the Negative Control (-), one microwell for the Blank (2 diluted to 1X), and two microwells for the Calibrator Cutoff (4). Appropriate controls must be treated prior to testing. Do <u>not</u> treat the Blank.
Step 3	Prepare 1X Wash Buffer (2 diluted to 1X with distilled or deionized water). This will be used for the blank and wash buffer.
Step 4	Treat controls (AGMCC1 4, AGMPC1 +, and AGMNC1 -) and samples using the Pretreatment Buffer ***(3) according to "Pretreatment of Controls, Serum and BAL" instructions listed above. Do <u>not</u> pretreat the blank.***
Step 5	Following completion of sample pretreatment and preparation, 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 (-) • Calibrator Cutoff (4) - 2 wells

	• Patient Samples Record the position of each Control, Blank, and Sample.
Step 6	Cover plate with plate sealer, or other means to prevent evaporation, ensuring the entire surface is covered and watertight.
Step 7	Incubate plate at 37°C ±1°C for 60 minutes ±5 minutes.
Step 8	Remove the plate sealer and, using a pipettor, aspirate the contents from the microwells and discard into a biohazard receptacle, changing tips between microwells.
Step 9	Using an EIA plate washer or multichannel pipettor, fill all microwells with 300 µL of 1X Wash Buffer (2) prepared in Step 3. Dump the plate contents after filling. Repeat 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 (5) to each microwell.
Step 12	Cover plate with plate sealer, or other means to prevent evaporation, ensuring the entire surface is covered and watertight.
Step 13	Incubate plate at 37°C ±1°C for 30 minutes ±5 minutes.
Step 14	Remove the plate sealer and, using a pipettor, aspirate the contents from the microwells and discard into a biohazard receptacle, changing tips between microwells.
Step 15	Using an EIA plate washer or multichannel pipettor, fill all microwells with 300 µL of 1X Wash Buffer (2) prepared in Step 3. Dump the plate contents after filling. Repeat for a total of 5 washes.
Step 16	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 17	Add 100 µL of Substrate (6) to each microwell. Start a timer for 30 minutes (±5 minutes) when 6 is added to the first microwell.
Step 18	Cover plate with plate sealer, or other means to prevent evaporation, ensuring the entire surface is covered and watertight.
Step 19	Incubate at 37°C ±1°C for the remainder of the 30-minute timer.
Step 20	Remove plate sealer and add 100 µL of Stop Solution (7) to each microwell in the same order as Step 17.
Step 21	Read and record results (see "Reading the Test"). *(NOTE: Reading should take place within 15 minutes.)*

READING THE TEST

A. **Results read after the 15-minute reading window are invalid.**
B. Mix by gently tapping the side of the plate or shaking on the countertop for 1-5 seconds.
C. Carefully wipe the undersides of the microwells with a clean, lint-free tissue.
D. Read the optical density of each microwell at both 450 nm and 620/630 nm. Blank on the 1X Wash Buffer (2 diluted to 1X).

1. A dual wavelength reader is required, with absorbances at 450 nm and 620/630 nm. This assay has not been validated with a single wavelength reader or at any other reference wavelengths.

E. Results must be read within 15 minutes of adding Stop Solution.
F. Discard any used assay materials as hazardous waste and retain microwell holder.
G. 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: *Calculations* and *Expected Results* can be found under "Quality Control & Results".

MATERIALS PROVIDED

See REAGENTS section

MATERIALS REQUIRED BUT NOT PROVIDED

A. Distilled or deionized water, for dilution of concentrated wash buffer
B. Absorbent paper
C. Timer
D. Vortex agitator
E. Pipettors capable of delivering ranges from 100-300 µL and disposable tips
F. Plate sealers or other means to prevent evaporation
G. 1.5-2.0 mL microcentrifuge screw-cap tubes (IMMY Ref# SCT050 or equivalent) able to support heating to 120°C (heat block) or 100°C (boiling water bath) for treatment of samples
H. Heat block capable of reaching 120°C or water bath capable of reaching 100°C for treatment of samples
I. Laboratory centrifuge for 1.5-2.0 mL tubes capable of withstanding 10,000-14,000 x g
J. Incubator set to 37 °C
K. Microplate washer or multichannel pipettor for washing
L. Microplate reader capable of reading absorbances at 450 and 620/630 nm

QUALITY CONTROL & RESULTS

1. Quality Control:

An assay is considered valid when the Calibrator Cutoff (CC), Positive Control (PC), Negative control (NC), and Blank (1X Wash Buffer) fall within the acceptable ranges, as defined in the tables located in the *Expected Results* section.

The Positive Control, Negative Control, Calibrator Cutoff, and Blank 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 precision. If the results of the Positive Control and/or Negative Control and/or Calibrator

Cutoff and/or Blank is not within these parameters, patient test results should be considered invalid, and the assay should be repeated with a new set of pretreated controls and samples.

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

2. Calculations:

Calculate EIA Units by the following:

1. Calculate the Corrected ODs of the controls, blank, and patient samples from the raw data:
$$Corrected\ OD = Sample\ OD\ 450nm - Sample\ OD\ 630nm$$
2. Calculate the Blanked ODs of the controls, blank, and patient samples from the Corrected ODs:
$$Blanked\ OD = Sample\ Corrected\ OD - Corrected\ Blank\ OD$$
3. Calculate the average value (mean) for the two Calibrator Cutoff microwells using the Blanked ODs.
4. Calculate sample EIA Units:

$$EIA\ Units = \frac{Blanked\ Sample\ OD}{Mean\ Blanked\ Calibrator\ Cutoff\ OD}$$

3. Expected Results:

CONTROLS	ACCEPTABLE VALUES
Calibrator Cutoff	0.150 – 0.300 Blanked OD
PC	2.5 – 4.0 EIA Units
NC	≤0.1 EIA Units
Blank	≤0.06 Corrected OD

RESULTS	EIA UNITS
Negative	<0.20 EIA Units
Positive	≥0.20 EIA Units

LIMITATIONS

A. A negative result does **not** exclude diagnosis of aspergillosis.
B. AGM101 is intended for use with serum and BAL specimens. This assay has not been validated on specimens other than serum and BAL.
C. Performance of reagents has not been determined when exposed to temperature fluctuations beyond those required by the test procedure.
D. Use only protocols described in this package insert. Incubation times or temperatures other than those specified may give inaccurate results.

E. The performance of AGM101 has not been established for manual reading and/or visual result determination.
F. Inadequate washing during the test procedure can cause excessive background reactivity.
G. 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.
H. Positive tests should be confirmed in areas or patient groups where organisms that are known to cross-react with *Aspergillus spp*. are endemic or a risk. Some cross-reactivity was observed with *Histoplasma* positive samples and therefore should be considered in endemic areas, including parts of the United States.
I. AGM101 was not evaluated for cross-reactivity using BAL specimens.
J. AGM101 was not evaluated for the potential of interference using BAL specimens.
K. AGM101 may exhibit reduced detection of galactomannan in patients with chronic granulomatous disease (CGD) and Job's syndrome.[12,13]
L. Fungal organisms such as *Talaromyces marneffei*, *Candida*, *Blastomyces*, *Histoplasma* and *Cryptococcus* are known to cross-react with other galactomannan assays[14]. AGM101 has not been evaluated for cross-reactivity against other fungi, except for *Histoplasma*.
M. AGM101 has not been evaluated for cross-reactivity with PLASMA-LYTE™. There have been reports of positive reactions for galactomannan associated with PLASMA-LYTE™ in several observations due to cross-reactivity or contamination issues[15-17]. Therefore, any administration of PLASMA-LYTE™ should be considered when interpreting the results of this test.
N. Cross-reactivity of BAL fluid samples with *Mycoplasma pneumoniae* or anesthetic drugs/lubricants used to numb the neck/throat area for the aspiration process has not been evaluated.
O. AGM101 has not been evaluated for cross-reactivity with Bifidobacterial lipoglycan.[18]
P. AGM101 is not intended for monitoring therapy.
Q. The use of mold-active anti-fungal therapy in some patients with Invasive Aspergillosis may result in reduced sensitivity with AGM101.
R. 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 aspergillosis disease being present. Testing should only be done when clinical evidence suggests the diagnosis of aspergillosis.
S. Results between different *Aspergillus* galactomannan assays cannot be compared.

PERFORMANCE CHARACTERISTICS

1. Expected Values

The frequency of aspergillosis is dependent on several factors including patient population, type of institution, and epidemiology. The expected prevalence of invasive aspergillosis in immunocompromised patients can vary from 5 – 20%[19].

2. Analytical Sensitivity (C95)

The clarus AGM EIA was evaluated for analytical sensitivity by spiking serum and BAL fluid with *Aspergillus* galactomannan antigen at 3.00 ng/mL and testing 8x serially diluted (1:2) concentrations. Each of the Serum concentrations were tested a minimum of 27 replicates, while the BAL fluid concentrations were tested for a total of 10 replicates (each).
The Analytical Sensitivity was determined by finding the intercept where 95% of the results were positive and is approximately 0.40-0.50 ng/mL for both Serum and BAL fluid.

3. High-Dose Hook Effect

High-Dose hook-effect was evaluated internally by testing human serum and BAL spiked with *Aspergillus* GM Antigen on the clarus AGM EIA. The AGM EIA did not exhibit complete hook-effect (false-negative results) when testing serum and BAL samples containing extremely high concentrations (up to 260 µg/mL).

SAMPLE CONCENTRATION	MEAN EIA UNITS	RESULT
260 µg/mL BAL	11.810	Positive
130 µg/mL BAL	11.852	Positive
65 µg/mL BAL	11.664	Positive
32 µg/mL BAL	11.975	Positive
260 µg/mL Serum	12.487	Positive
130 µg/mL Serum	12.565	Positive
65 µg/mL Serum	12.633	Positive
32 µg/mL Serum	12.280	Positive

4. Interference

The clarus AGM EIA was evaluated for the potential of interference on hemolyzed, icteric, and lipemic serum. These sera exhibit no interference in the assay.

5. Cross-Reactivity

The clarus AGM EIA was evaluated for cross-reactivity against a panel of patient serum specimens across a variety of different pathologies. The results of this testing are shown in the table below:

PATHOLOGY	NUMBER OF SPECIMENS	% POSITIVE
Syphilis	10	0% (0/10)
Toxoplasmosis	8	0% (0/8)
HAV	10	0% (0/10)
ANA	10	0% (0/10)
Rubella	10	0% (0/10)
CMV	5	0% (0/5)
Rheumatoid Factor	10	0% (0/10)
Mycoplasma	10	0% (0/10)

PATHOLOGY	NUMBER OF SPECIMENS	% POSITIVE
HCV	10	0% (0/10)
*Cancer**	15	0% (0/15)
*Histoplasma***	8	12% (1/8)

*Cancer types evaluated: 1x Sarcoma, 5x Lymphomas, 1x Neuroblastoma, 5x Myeloma, 1x Lung Cancer, and 1x Renal Cancer

** The single *Histoplasma* cross-reactive serum specimen was also positive on another commercially available *Aspergillus* GM EIA, with a GM Index of 7.524, compared to 0.498 EIA Units on the clarus *A*GM101. Notably, a second *Histoplasma* serum specimen was also positive (2.269 GM Index) on the same commercially available GM EIA, resulting in a *Histoplasma* cross-reactivity rate of 25% (2/8), compared to 12% for the clarus AGM101.

6. Precision

Precision (Reproducibility) on the clarus AGM EIA was evaluated by running controls and a panel of three serum and four BAL specimens. The specimen panel was tested once a day for a total of five days, in two different lots, by three operators (totaling 30 evaluations per sample). Samples tested: Assay controls (AGMCC1, AGMPC1, AGMNC1, 1x Wash-Buffer/Blank), two low positives (1x Serum, 1x BAL), two moderate positives (1x Serum, 1x BAL), and three negatives (1x Serum, 2x BAL).

For precision, the mean, standard deviation, %CV, % Positive and % Negative were calculated for the EIA Units results. The results are shown in the table below:

SAMPLE TYPE	MEAN (EIA UNIT)	SD	%CV	% POSITIVE	% NEGATIVE
Mod. Positive Serum	0,834	0,082	10%	(30/30) 100%	(0/30) 0%
Low Positive Serum	0,443	0,041	9%	(28/28) 100%	(0/28) 0%
Mod. Positive BAL	0,614	0,086	13%	(30/30) 100%	(0/30) 0%
Low Positive BAL	0,293	0,051	17%	(30/30) 100%	(0/30) 0%
Negative BAL 1	0,039	0,037	N/A	(0/30) 0%	(30/30) 100%
Negative BAL 2	-0,008	0,035	N/A	(0/30) 0%	(30/30) 100%
Negative Serum	0,003	0,038	N/A	(0/30) 0%	(30/30) 100%

7. Method Comparison

The clarus AGM EIA was compared to a commercially available *Aspergillus* GM Antigen EIA in an external study using retrospective specimens. A total of 319 specimens (272 serum, 47 BAL) were tested on both assays. 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:

Serum		GM EIA	
		Pos.	Neg.
IMMY clarus AGM EIA	Pos.	32	1
	Neg.	13	226

Serum	Calculated	95% CI
% Pos. Agreement	71.1%	56.6%-82.3%
% Neg. Agreement	99.6%	97.5%-99.9%

BAL Fluid		GM EIA	
		Pos.	Neg.
IMMY clarus AGM EIA	Pos.	16	1
	Neg.	3	27

BAL Fluid	Calculated	95% CI
% Pos. Agreement	84.2%	62.4%-94.5%
% Neg. Agreement	96.4%	82.3%-99.4%

8. Clinical Performance

An external study based on the 2020 EORTC/MSG Clinical Criteria for IA was performed to determine the sensitivity and specificity of the clarus AGM EIA by evaluating 290 retrospective specimens (254 serum, 36 BAL Fluid) from patients at risk for IA. The results of this comparison are shown in the tables below:

Serum		EORTC/MSG	
		Proven/ Probable	No IA
IMMY clarus Asp. GM EIA	Pos.	32	0
	Neg.	6	216

Serum	Calculated	95% CI
Sensitivity	84.2 %	69.6%-92.6%
Specificity	100.0%	98.3%-100%

BAL Fluid		EORTC/MSG	
		Proven/ Probable	No IA
IMMY clarus Asp. GM EIA	Pos.	14	1
	Neg.	2	19

BAL Fluid	Calculated	95% CI
Sensitivity	87.5%	64.0%-96.5%
Specificity	95.0%	76.4%-99.1%

9. Reference Procedures and Materials

There are no available reference measurement procedures or materials for the user.

TROUBLESHOOTING

PROBLEM	SOLUTION
Variable results across replicates	• Set-up controls and samples in a separate, clean, non-coated 96-well plate • Use multichannel to pipette from the 96-well plate into the microwells

PROBLEM	SOLUTION
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
Lower ODs than expected (Reagents too cold)	• Make sure all reagents come to 18-25°C before testing

SYMBOLS

	Contains sufficient for <*n*> tests		Catalogue number/Reference number
	Consult instructions for use		Batch code/Lot code
	Manufacturer		For In Vitro Diagnostic Use
	Use-by date/Expiration date		Temperature limit
	Positive Control		CE Mark Of Conformity
	Negative Control		Authorized representative in the European Community/European Union
	Single Use Only		

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IMMY, Inc.
2701 Corporate Centre Drive
Norman, OK 73069 U.S.A
(405) 360-4669/(800) 654-3639
Fax: (405) 364-1058
E-mail: info@immy.com
Web: www.immy.com

EC REP
MDSS
Schiffgraben 41
30175 Hannover, Germany