

INTENDED USE

The sōna *Aspergillus* Galactomannan Lateral Flow Assay (AGM LFA) is an immunochromatographic test system for the qualitative detection of *Aspergillus* galactomannan in serum and bronchoalveolar lavage (BAL) samples.

The sōna AGM LFA is a test which can be used as an aid in the diagnosis of aspergillosis when used in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples, and radiographic evidence.

SUMMARY AND EXPLANATION OF THE TEST

Aspergillus spp. are 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 high risk¹⁻³. 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^{4,5}. 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 population². Early detection and treatment of infection are key to reducing the mortality associated with this disease^{6,7}.

BIOLOGICAL PRINCIPLES

The sōna AGM LFA is a sandwich immunochromatographic test system which detects *Aspergillus* galactomannan in serum and BAL specimens. Serum and BAL specimens require heat pretreatment prior to testing. After pretreatment, specimens are pipetted into a clean receptacle. *Aspergillus* GM LFA Running Buffer (REF AFLFRB) is added followed by an *Aspergillus* GM Lateral Flow Test Strip (REF LFAF50). The test is run for 30 minutes and results should be read within 10 minutes of completing the test.

The LFA is constructed by having *Aspergillus* galactomannan specific antibodies conjugated to colloidal gold that bind to any galactomannan that may be present in the specimen sample as it wicks up the test strip. If any binding occurs, the antibody-antigen complex will migrate up the strip by capillary flow until it is captured by the *Aspergillus* galactomannan specific antibodies in the test line. This results in the formation of a visible test line. Additionally, control antibodies conjugated to gold are present that wick along with the specimen and will be captured by the control antibodies present on the control line, regardless of positive or negative test results.

To read visually, positive test results create two lines (test and control lines) and negative results form only one line (control line). If a control line fails to develop, the test is invalid.

The IMMY Cube Reader is an optional accessory to aid in the interpretation of the sōna AGM LFA. The Cube Reader uses an LED at 525 nm to read results on the sōna AGM LFA. Index values ≥ 0.50 are considered positive and will display as POS. Index values < 0.50 are considered negative and will display as NEG. Invalid results will read as INV.

REAGENTS

1. **Sample Pretreatment Buffer (7 mL) (REF AFSPB1) [1]** – EDTA solution containing a preservative.
2. ***Aspergillus* GM Running Buffer (3 mL) (REF AFLFRB) [2]** – LFA running buffer containing a preservative.
3. ***Aspergillus* GM Lateral Flow Test Strips (50 each) (REF LFAF50) [3]** – LFA dipstick packaged into a desiccant vial with an attached cap.
4. ***Aspergillus* GM Positive Control (3 mL) (REF AFPC01) [4]** – *Aspergillus* galactomannan in a saline solution containing a preservative.
5. **Package Insert.**

Refer to product Safety Data Sheets for more information on hazards and warnings.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable gloves
2. Protective glasses
3. Pipette(s) capable of measuring and delivering 300, 100, 80, and 40 µL and associated disposable tips
4. 1.5-2.0 mL microcentrifuge screw cap tubes able to support heating up to 120 °C (heat block)
5. Heat Block capable of reaching 120 °C
6. Centrifuge capable of reaching 14,000 x g
7. Disposable flat-bottom micro-centrifuge tubes, test tubes, or a micro-titer plate
8. Vortex mixer
9. Timer

REAGENT STABILITY AND STORAGE

The entire sōna AGM LFA test kit should be stored at 2-30 °C until the expiration date listed on the outside of the kit label. At the time of each use, kit components should be visually inspected for obvious signs of microbial contamination, freezing or leakage. Discard if these conditions are found.

Unused test strips should be stored in the LF test strip vial with the cap firmly closed.

WARNINGS FOR USERS

1. For In Vitro Diagnostic use only.
2. For professional use only.
3. Use of this kit with samples other than human serum and BAL fluid is not recommended.
4. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
5. Avoid splashing samples or solutions.
6. 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 0.5% Wescodyne Plus™. Materials used to wipe up spills may require biohazardous waste disposal.
7. 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.
8. The *Aspergillus* GM Lateral Flow Test Strips (REF LFAF50) may be biohazardous after running specimens. Handle and dispose of accordingly.
9. Refer to Hazards and Precautionary Information section for hazards associated with specific reagents. Safety Data Sheets are available upon request.
10. Results read after the 10-minute reading window are invalid.

PRECAUTIONS FOR USERS

1. **FROZEN SERUM OR BAL SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE FALSE POSITIVE RESULTS DUE TO CONTAMINATION WITH FUNGI AND/OR BACTERIA.**
2. Do not use kit or any kit reagents after the stated expiration date.
3. Use clean, dust-free materials (tubes, tips, containers, etc.) 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.
4. Limit exposure of samples and kit components (sera, BAL fluid, Sample Pretreatment buffer, Running Buffer, Test Strips) or open containers (plates, tubes, pipette tips) to the air.
5. Heat block temperature should be confirmed by a separate thermometer to independently assess actual heat block temperature.
6. 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.
7. If sample has inadequate volume for testing (80 µL) after pretreatment, repeat pretreatment steps with a fresh sample. Incomplete pretreatment may lead to erroneous results.

REAGENT PRECAUTIONS

1. Specific standardization is necessary to produce our high-quality reagents and materials. 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.
2. The user assumes full responsibility for any modification to the procedures published herein.

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, sterile samples should be used. Process and test samples upon arrival. If a delay is encountered in specimen processing, storage for up to 5 days at 2-8 °C or up to 2 weeks at < -20 °C is permissible. However, a very low-positive specimen could become negative after storage. Specimens in transit between labs should be maintained at 2-8 °C. Specimens should be brought to room temperature prior to testing.

SPECIMEN PREPARATION

Pretreatment of Serum and BAL:

1. Place 300 µL of fresh serum or BAL into a screw cap, heat resistant microcentrifuge tube.
2. Add 100 µL of Sample Pretreatment Buffer [1] to the same tube.

3. Screw the lid on tightly and vortex the sample.

4. Place tube in a heat block for 6-8 minutes at 120 °C.
Note: Use calibrated thermometer to determine temperature of heat block. Do not rely on heat block temperature display as it may not be accurate.

5. Immediately centrifuge sample for 5 minutes at 10,000-14,000 x g at room temperature.

6. After pretreatment, treated sample can be stored at 2-8 °C for up to 7 hours prior to testing. If sample analysis requires retesting, a separate aliquot of the sample must be pretreated for retesting.

PROCEDURE

1. Add 120 µL of *Aspergillus* GM Positive Control [4] into a clean tube or microwell and add 120 µL of *Aspergillus* GM LFA Running Buffer [2] [Negative Control] into another clean tube or microwell. It is recommended that controls be tested monthly. **Note: Do not boil negative and/or positive controls.**

2. Pipette 40 µL of *Aspergillus* GM LFA Running Buffer [2] into a separate clean tube or microwell.

3. Pipette 80 µL of supernatant from pretreated serum/BAL to each tube or microwell from step 2.

4. Place one *Aspergillus* GM Lateral Flow Test Strip [3] in each tube or microwell containing a sample or control.

5. Allow the test to run for 30 minutes at room temperature.

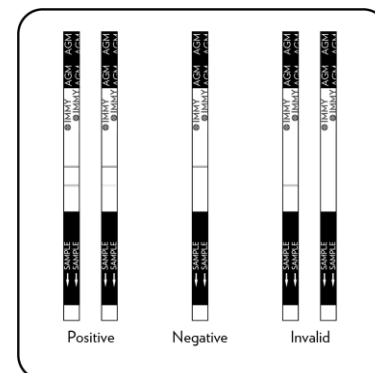
6. Read and record results within 10 minutes of completing the test either visually or with the Cube Reader.

READING THE TEST

Results read after the 10-minute reading window are invalid.

For Visual Read: Read the reactions. The presence of two pink lines (test and control), regardless of the intensity of the test line, indicates a positive result. A single control line (top line) indicates a negative result. If the control line does not appear, the results are invalid, and the test should be repeated.

For Visual Interpretation of Results



2 lines = positive | 1 line = negative

For Interpretation using Cube Reader: Results ≥ 0.50 are considered positive and will display as POS. Results < 0.50 are considered negative and will display as NEG. Invalid results will read as INV.

1. Run the sōna *Aspergillus* GM LFA according to product Package Insert.

2. Press the button on the top of the sōna LFA Cube Reader twice until display reads "RFID".

3. Scan the lot-specific RFID tag located on the bottom of the *Aspergillus* GM Lateral Flow Test Strip Tube (REF# LFAF50) by placing it over the display on the cube reader. An audible signal will confirm scanning of RFID tag and "TEST" will appear on display.

4. When the test strip is ready to be analyzed, properly insert the LFA strip into cube reader so the sample arrows of the strip are facing the same direction as the sample arrows on the adapter itself. Always ensure the LFA strip is positioned flush against the left side of the slot in the adapter, following the sample arrow. If not correctly placed the test results may be invalid. Results should be read within 10 minutes of completion of the test's 30-minute incubation.

5. While "TEST" is still displayed on the cube reader, press the button once to run. "RUN" will appear on display while the strip is being read.

6. Results will display as a numerical value for the test line, followed by "POS" or "NEG", followed by a numerical value for the control line. Record displayed test results.

7. To test another strip of the same lot, remove the strip and press the button on the Cube Reader three times until "TEST" appears on display. Repeat steps 4-6.

Cut-Off for Results with Cube Reader

Result	Index Value
Positive – POS	≥ 0.50
Negative – NEG	< 0.50
Invalid – INV	N/A

QUALITY CONTROL

Positive and negative controls verify the kit is working as intended and ensure no product failure or no contamination has occurred. A positive control (*Aspergillus* GM Positive Control **1**) can be evaluated by adding 120 µL to a tube. A negative control can be evaluated by adding 120 µL of *Aspergillus* GM LFA Running Buffer (REF **2**) to a separate tube. Insert a test strip into the tubes and read after 30 minutes.

If reading visually, two (2) lines (test and control) indicate a positive result and one line (control) indicates a negative result.

If using the Cube Reader, the positive control should produce an index value of ≥ 0.50 and the negative control should produce an index value of < 0.50. Invalid results will read as INV.

Recommended Quality Control frequency is 1 time per run. The lack of a visible control line or a weak control line can be indicative of an incomplete pretreatment. Slight variation in control line intensity is normal and is dependent upon the intensity of the test line. If controls produce results different than above, contact IMMY Customer Support.

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

INTERPRETATION OF RESULTS

The control line must be present for a valid test. If reading visually, the presence of two lines (a control line and a line in the test zone) indicates a positive result. The presence of one line (control line) indicates a negative result.

For interpretation with the Cube Reader, results ≥ 0.50 are considered positive and will display as POS. Results < 0.50 are considered negative and will display as NEG.

Negative results do not rule out the diagnosis of disease. The specimen may be drawn before detectable antigen is present.

LIMITATIONS OF THE PROCEDURE

- The assay performance characteristics have not been established for matrices other than serum and BAL fluid.
- Depending on the disease and organism prevalence, testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of aspergillosis disease being present. Testing should only be done when clinical evidence suggests the diagnosis of aspergillosis disease.
- Testing hemolyzed serum samples could lead to false negatives due to the high background color on the strip.
- 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.
- Sufficient contact between the screw cap tube and the heat block must be maintained throughout the boil during the pretreatment step. Contact IMMY Customer Service for assistance and for further information.

CROSS-REACTIVITY ANALYSIS

The *sōna Aspergillus* Galactomannan Lateral Flow Assay was evaluated for cross-reactivity against a panel of patients' sera specimens across a variety of different pathologies. The results of this testing are shown in the table below.

Note: Galactomannan EIA results are unknown. Specimens may be positive by the EIA.

Pathology	# of Samples	% Positive
ANA-Positive	1	0% (0/1)
Syphilis	3	0% (0/3)
Rubella	2	0% (0/2)
Mycoplasma	2	0% (0/2)
Toxoplasmosis	3	0% (0/3)
CMV Infection	3	0% (0/3)
Rheumatoid factor	3	0% (0/3)
Hepatitis C Virus	2	0% (0/2)
Cancer	5	20% (1/5)
Solid Organ Transplant	5	0% (0/5)

Additionally, cross-reactivity was assessed by testing infections of other fungal pathogens using the AGM LFA. Cross-reactivity was observed with some histoplasmosis, candidiasis, and coccidioidomycosis specimens.

Characterized specimens of the following infections were tested and exhibited no cross-reactivity: blastomycosis and cryptococcosis.

HIGH DOSE HOOK EFFECT (PROZONING)

Although rare, extremely high concentrations (>0.225 mg/mL) of *Aspergillus* galactomannan antigen can result in weak test and control lines.

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 varies from 5 – 20% (8).

SPECIFIC PERFORMANCE CHARACTERISTICS

The *sōna* AGM LFA was compared to EORTC/MSG clinical criteria to show sensitivity and specificity. These studies contained prospective

and retrospective specimens that were submitted for Asp Ag EIA testing. Summary tables of the data collected are included below.

	Visual			
	Serum Sens.	Serum Spec.	BAL Fluid Sens.	BAL Fluid Spec.
Point Estimate	100%	97%	78%	82%
95% CI	16-100%	92-99%	72-83%	77-87%

	Cube Reader			
	Serum Sens.	Serum Spec.	BAL Fluid Sens.	BAL Fluid Spec.
Point Estimate	97%	96%	100%	39%*
95% CI	85-100%	92-98%	3-100%	20-61%

*An EIA for detection of *Aspergillus* antigen had a specificity of 44% using the same dataset

REPRODUCIBILITY AND PRECISION

The *sōna* AGM LFA was evaluated for reproducibility and precision by spiking serum and artificial BAL (aBAL) with *Aspergillus* galactomannan antigen to produce 5 panels consisting of negative samples, low-positive samples, and moderate-positive samples. Five operators, blinded to sample identity, tested each of the five panels each day over the course of 5 days. Results were read both visually and with the Cube Reader. The results of this study are shown in the tables below.

SERUM	Visual		Cube Reader	
	# Correct	% Correct	# Correct	% Correct
Neg	63	84%	73	99%
Low Pos	48	64%	52	69%
Mod Pos	74	96%	74	100%

BAL	Visual		Cube Reader	
	# Correct	% Correct	# Correct	% Correct
Neg	60	82%	73	100%
Low Pos	68	91%	72	96%
Mod Pos	75	100%	74	99%

CLEANING THE CUBE READER

- Remove the *sōna* LFA Cube Reader from the adapter by gently applying downwards pressure on the adapter tab and lifting the cube reader out of adapter.
- Clean the LFA Cube Reader Adapter with a disinfectant. See Warnings for Users.
- Clean the cube reader lens with a lint-free cloth.
- Place the cube reader back in the adapter by matching the angled corner of the cube reader with the angled corner of the cube reader adapter. Gently apply downwards pressure to the adapter tab and insert the cube reader, backside first. Press the cube reader firmly into place and release adapter tab. The cube reader should be firmly seated into the adapter before use.

HAZARDS AND PRECAUTIONARY INFORMATION

Hazardous components

- AFPC01 **1**), AFLFRB **2**) Contain Boric Acid

Signal word: Danger



Hazard Statement(s)	
H360	May damage fertility or the unborn child.
Precautionary Statement(s)	
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P308 + P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.
P501	Dispose of contents/container in accordance with local regulations.

BIBLIOGRAPHY

- Barnes PD, Marr KA. Aspergillosis: spectrum of disease, diagnosis, and treatment. *Infect Dis Clin North Am.* 2006;20(3):545-61.
- Singh N, and Paterson DL. Aspergillus infections in transplant recipients. *Clin Microbiol Rev.* 2005;18(1):44-69.
- Soubani AO, Qureshi MA. Invasive pulmonary aspergillosis following bone marrow transplantation: risk factors and diagnostic aspect. *Haematologia (Budap).* 2002;32(4):427-37.
- Chamilos G, Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period(1989-2003). *Haematologica.* 2006;91(7):986-9.
- McNeil MM, Nash SL, Hajjeh RA, Phelan MA, Conn LA, Plikaytis BD, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. *Clin Infect Dis.* 2001;33(5):641-7.
- Mercier T, Dunbar A, de Kort E, Schauwvlieghe A, Reynders M, Guldentops E, et al. Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: A comparative multicenter study. *Med Myc.* 2019;1-9.
- van der Peppel RJ, Visser LG, Dekkers OM, de Boer MGJ. The burden of Invasive Aspergillosis in patients with haematological malignancy: A meta-analysis and systematic review. *Journal of Infect.* 2018;76(6):550-562.
- Denning DW. Invasive Aspergillosis. *Clin Infect Dis.* 1998; 26:781-803
- Zilberberg MD, Nathanson BH, Harrington R, Spalding JR, Shorr AF. Epidemiology and Outcomes of Hospitalizations with Invasive Aspergillosis in the United States, 2009-2013. *Clin Infect Dis.* 2018; 67(5):727-735.

INTERNATIONAL SYMBOL USAGE

	Storage 2-30 °C		Lot Number
	Manufactured by		Reference Number
	Expiration Date		In Vitro Diagnostic
	Protect from Humidity		Sufficient for "#" Tests

Rev. Date 2021-08-11
Rev. 8

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