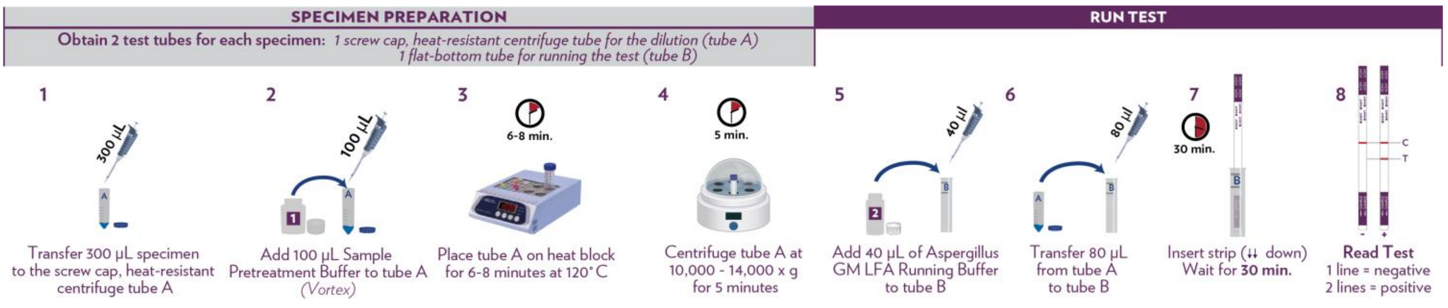
IMMY

sōna *Aspergillus* Galactomannan LFA

For the Detection of *Aspergillus* Galactomannan – REF AF2003



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 risk[1-3]. 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 population[2]. 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. 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.

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)** [+] – *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.

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 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 (+/- 3°C).
5. Immediately centrifuge sample for 5 minutes at 10,000-14,000 x g at room temperature.
6. After pretreatment, sample supernatant can be removed and stored at 2-8°C for up to 6 hours prior to testing. If sample analysis requires retesting, a separate aliquot of the sample must be pretreated for retesting.

PROCEDURE

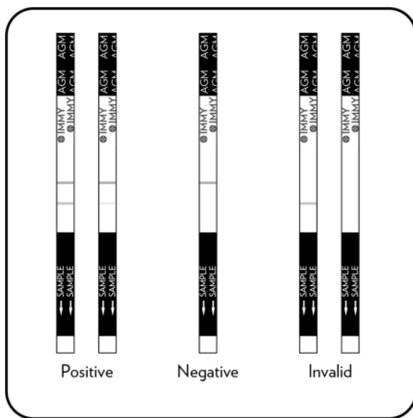
1. Add 120 µL of *Aspergillus* GM Positive Control (+) into a clean tube or microwell and add 120 µL of *Aspergillus* GM LFA Running Buffer (2) [Negative Control] into another clean tube or microwell. It is recommended that controls be tested monthly. **Note:** *Do not boil negative and/or positive controls.*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

READING THE TEST

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.

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

Interpretation of Results:



2 lines = positive | 1 line = negative

QUALITY CONTROL

Positive and negative controls verify the kit is working as intended and ensure no product failure or contamination has not occurred. A positive control (*Aspergillus* GM Positive Control +) 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. Two (2) lines (test and control) indicate a positive result and one line (control) indicates a negative result. 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. 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.

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.

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. Each specimen was tested multiple times. The results of this testing are shown in the table below.

Note: *Galactomannan EIA results are unknown. Specimens may be positive by the EIA. Specimens were purchased through a commercial entity so specimen sterility is unknown.*

Pathology	# of Samples	Total # of Tests	% Positive
ANA-Positive	13	38	0% (0/38)
Syphilis	16	45	4.4% (2/45)
Rubella	4	16	0% (0/16)
Mycoplasma	15	39	5.1% (2/39)
Toxoplasmosis	13	39	0% (0/39)
CMV Infection	9	27	0% (0/27)
Rheumatoid factor	30	58	0% (0/58)
Hepatitis A Virus	9	12	0% (0/12)
Hepatitis C Virus	10	13	0% (0/13)
Cancer	10	10	0% (0/10)

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the AGM LFA. At high concentrations (>0.1 mg/mL) antigens from *Paracoccidiodes brasiliensis, Coccidioides, Histoplasma, and Candida* exhibited some cross-reactivity.

Antigens from the following organisms were tested and exhibited no cross-reactivity: *Blastomyces, Cryptococcus*

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 a commercially available *Aspergillus* Ag EIA. These studies contained retrospective specimens that were submitted to a clinical laboratory for Asp Ag EIA testing. All studies were performed using previously frozen samples. Summary tables of the data collected are included below.

Serum		Asp Ag EIA	
		Positive	Negative
AGM LFA Assay	Positive	26	1
	Negative	6	116

Serum	Calculated	95% CI
% Agreement Pos	81%	64% - 93%
% Agreement Neg	99%	95% - 99.9%

BAL		Asp Ag EIA	
		Positive	Negative
AGM LFA Assay	Positive	25	3
	Negative	3	48

BAL	Calculated	95% CI
% Agreement Pos	89%	72% - 98%
% Agreement Neg	94%	84% - 99%

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 a panel consisting of a negative sample, a high-negative (C_5) sample, a low-positive sample, a moderate-positive sample, and a high positive (C_{95}) sample. This panel was tested in triplicate, daily, for 5 days at one site. The results of this study are shown in the tables below.

SERUM	# Positive	% Positive	# Negative	% Negative
Neg	0	0%	30	100%
High Neg	1	7%	14	93%
Low Pos	30	100%	0	0%
Mod Pos	30	100%	0	0%
High Pos	30	100%	0	0%

aBAL	# Positive	% Positive	# Negative	% Negative
Neg	0	0%	30	100%
High Neg	1	7%	14	93%
Low Pos	30	100%	0	0%
Mod Pos	30	100%	0	0%
High Pos	30	100%	0	0%

HAZARDS AND PRECAUTIONARY INFORMATION

Hazardous components

- AFPC01 (+), 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.

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INTERNATIONAL SYMBOL USAGE

Symbol	Meaning	Symbol	Meaning
2°C–30°C	Storage 2-30 °C	LOT	Lot Number
	Manufactured by	REF	Reference Number
	Expiration Date	IVD	In Vitro Diagnostic
	Protect from Humidity	Σ	Sufficient for "#" Tests

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Rev. 6