# sōna *Aspergillus* Galactomannan LFA

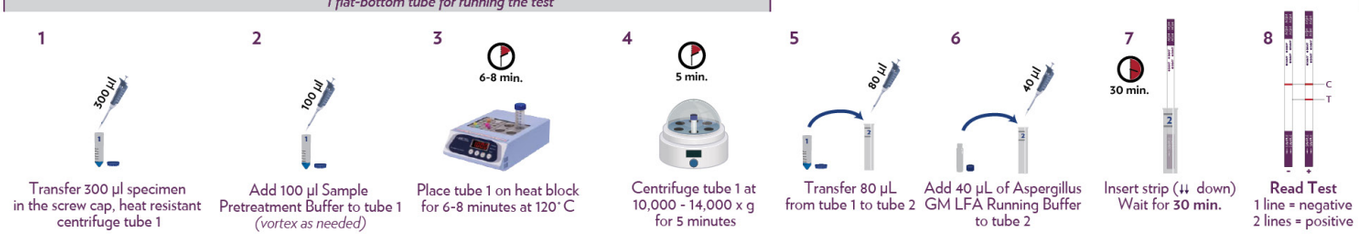
**For the Detection of *Aspergillus* Galactomannan – REF AF2003**

IVD CE 25° C 2° C

**SPECIMEN PREPARATION**

Obtain 2 test tubes for each specimen: *1 screw cap, heat reistant centrifuge tube for the dilution*
*1 flat-bottom tube for running the test*

**RUN TEST**




***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 BAL samples.

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

***SUMMARY AND EXPLANATION OF THE TEST***

*Aspergillus* spp. are ubiquitous filamentous fungus that are the causative agent of invasive aspergillosis (IA). IA is one of the most significant threats to recipients of hematopoietic stem cell and solid organ transplants, as well as, individuals with suppressed immune systems due to illnesses such as HIV/AIDS infection (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 has 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 pre-treatment prior to testing. After pre-treatment, Serum and BAL specimens are pipetted into a clean tube, a drop of Running Buffer (REF AFLFRB) is added, followed by an LFA strip (REF LFAF50). The test is run for 30 minutes and results can be read within 10 minutes of completing the test.

The LFA is constructed by having *Aspergillus* Galactomannan specific antibodies conjugated to colloidal gold that binds 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, resulting 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 not valid.

***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. Safety Data Sheets are available upon request.

***PRECAUTIONS FOR USERS***

1. **FROZEN SERUM OR BAL SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE FALSE POSITIVE RESULTS DUE TO CONTAMINATION WITH FUNGUS 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 Pre-treatment buffer, Running Buffer, Test strips) or open containers (plates, tubes, pipette tips) to the air.

***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.

***REAGENTS***

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

***MATERIALS REQUIRED BUT NOT PROVIDED***

1. Disposable gloves.
2. Protective glasses.
3. Pipette(s) capable of measuring and delivering up to 300 µL and associated disposable tips.
4. 1.5-2.0 mL microcentrifuge screw-capped tubes able to support heating up to 120 °C (heat block).
5. Heat block capable of reaching 120 °C.
6. Centrifuge capable of reaching 10,000 x g.
7. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate.
8. Vortex mixer.
9. Timer.

***REAGENT STABILITY AND STORAGE***

The entire *Aspergillus* Galactomannan LFA test kit should be stored at 2-25 °C until the expiration dates listed on the reagent labels. 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 attached.

***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***

**Treatment of Serum and BAL:**

1. Place 300 µL of fresh serum or BAL into a screw cap, heat resistant centrifuge tube.
2. Add 100 µL of Sample Pretreatment Buffer (REF AFSPB1) 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. Spin sample for 5 minutes at 10,000-14,000 x g at room temperature.

***TESTING PROCEDURES***

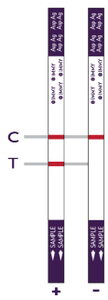
***PROCEDURE***

1. Add 120 µL of Positive Control (REF AFPC01) into a clean tube or microwell and 120 µL of AGM LFA Running Buffer (REF AFLFRB) [Negative Control] into a clean tube or microwell. It is recommended that controls be tested monthly.
   **Note:** *Do not boil negative and/or positive controls.*
2. Pipette 80 µL of supernatant from treated serum/BAL into a separate clean tube or microwell.
3. Pipette 40 µL of AGM LFA Running Buffer (REF AFLFRB) to each tube or microwell from step 2.
4. Place one AGM Lateral Flow Strip (REF LFAF50) test strip in each tube or microwell containing a sample or control.
5. Allow the test to run for 30 minutes at RT.
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.

**Interpretation of Results:**




2 lines = positive | 1 line = negative

### *QUALITY CONTROL*

A positive control (AGM Positive Control REF AFPC01) can be evaluated by adding 120µL to a tube. A negative control can be evaluated by adding AGM LFA Running Buffer (REF AFLFRB) to a 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.

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. Note: A gray line should be interpreted as negative.

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 fluids samples with *Mycoplasma pneumoniae* or anesthetic drugs/lubricants used to num the neck/throat area for the aspiration process has not been evaluated.

### *CROSS-REACTIVITY ANALYSIS*

The sōna AGM Lateral Flow Assay was evaluated for cross-reactivity against a panel of patients' serum 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 Lateral Flow Assay. 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 (>0.900 mg/mL) of *Aspergilllus* galactomannan antigen can result in weak test 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 Lateral Flow Assay 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. 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% |

### LIMIT OF DETECTION

In order to establish the limit of detection, a $C_5$- $C_{95}$ experiment was conducted by diluting purified *Aspergillus* galactomannan antigen in negative serum and BAL and testing 24 replicates per concentration using the sōna AGM Lateral Flow Assay. The results of this testing are shown in the following tables:

| Serum Concentration (ng/ml) | # Positive | % Positive |
|---|---|---|
| 0.6 | 0 | 0% |
| 0.7 | 0 | 0% |
| 0.8 | 5 | 21% |
| 0.9 | 11 | 46% |
| 1.0 | 12 | 50% |
| 1.1 | 14 | 58% |
| 1.2 | 16 | 67% |
| 1.3 | 17 | 71% |
| 1.4 | 19 | 79% |
| 1.5 | 20 | 83% |
| 1.6 | 22 | 92% |
| 1.7 | 24 | 100% |
| 1.8 | 24 | 100% |

| BAL Concentration (ng/ml) | # Positive | % Positive |
|---|---|---|
| 0.25 | 0 | 0% |
| 0.5 | 0 | 0% |
| 0.75 | 1 | 4% |
| 1.0 | 2 | 8% |
| 1.25 | 4 | 17% |
| 1.5 | 10 | 42% |
| 1.75 | 14 | 58% |
| 2.0 | 20 | 83% |
| 2.25 | 24 | 100% |
| 2.5 | 24 | 100% |

| | |
|---|---|
| Serum $C_5$ – $C_{95}$ Interval | 0.75 – 1.6 ng/mL |
| BAL $C_5$ – $C_{95}$ Interval | 0.75 – 2.1ng/mL |

### REPRODUCIBILITY AND PRECISION

The sōna AGM Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum and artificial BAL (aBAL) with *Aspergillus* GM 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 ($C_{95}$). 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% |
| Med 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% |
| Med Pos | 30 | 100% | 0 | 0% |
| High Pos | 30 | 100% | 0 | 0% |

### *BIBLIOGRAPHY*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**International Symbol Usage**

| | | | |
|---|---|---|---|
| | Storage 2-25 °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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