INTENDED USE

The sōna Aspergillus Galactomannan Lateral Flow Assay (AGMA) is an approximately 15-minute, portable test system for the qualitative detection of Aspergillus galactomannan in serum and bronchoalveolar lavage (BAL) samples.

The sōna AGMA LFA is a test which can be used as an aid in the diagnosis of IA. This test is in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy specimens, and serological tests.

SUMMARY AND EXPLANATION OF THE TEST

Aspergillus spp. are filamentous fungi found worldwide and can live in both indoors and outdoors. Invasive aspergillosis (IA) is caused by inhaling spores of these fungi. IA is a life-threatening disease to recipients of hematopoietic stem cell and solid organ transplants. Individuals with suppressed immune systems due to illnesses such as AIDS or cancer are also at high risk. 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. It has been reported that IA infections account for up to 44% of infections within transplant patients and have a staggering mortality rate of up to 92% within this population. Early detection and treatment of infection is key to reducing the mortality associated with this disease.

BIOLOGICAL PRINCIPLES

The sōna AGMA LFA is a sandwich immunochromatographic test system which is used to detect Aspergillus galactomannan in serum and BAL specimens. Serum and BAL specimens require heat pre-treatment prior to testing. After pre-treatment, specimens are pipetted into a clean receptacle. The sōna Aspergillus GM Lateral Flow Running Buffer (REF AFRLBF) is added followed by an Aspergillus GM Lateral Flow Test Strip (REF LFAF50). The test is run for 20 minutes and results should be read within 10 minutes of completion. The LFA is constructed by having Aspergillus galactomannan-specific antibodies conjugated to colloidal gold that bind to any galactomannan that is present in the test sample. The antibodies, sandwiched between two layers of paper, form a 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 colloidal gold are present that will run 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 form, the test should be repeated, as this may indicate instrument malfunction.

The sōna Cube Reader is an optional accessory to aid in the interpretation of the sōna AGMA LFA. The Cube Reader uses an LED at 520 nm to read results on the sōna AGMA 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 Pre-treatment Buffer (7 mL) (REF ASFBSP)
2. Aspergillus GM Running Buffer (REF AFRLBF) – LFA running buffer containing a preservative
3. Aspergillus GM Lateral Flow Test Strips (50 each) (REF LFAF50) – LFA dipstick packaged into a desiccant vial with an attached cap
4. Specific standardization is necessary to produce our high quality reagents and materials. sōna cannot guarantee the performance of its products when used with materials purchased from other manufacturers. Do not interchange reagents from different lot numbers or other manufacturers.
5. The user assumes full responsibility for any modification to the procedures published herein.

PRECAUTIONS FOR USERS

1. FROZEN SERUM OR BAL SAMPLES STORED IN UNKNOW CONDITIONS MAY GIVE FALSE POSITIVE RESULTS DUE TO CONTAMINATION WITH FUNGI AND/OR BACTERIA.
2. Do not use kit or any 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 contaminants. Pungent-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.
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 pre-treatment, for optimal reactively specimens should be centrifuged immediately.
7. If a 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 STABILITY AND STORAGE

The entire sōna AGMA 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 LIF 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. For accuracy, only human sera should be used.
4. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and patient specimens with extreme care to prevent exposure to biologically hazardous materials.
5. Blood spills should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% hypochlorite, 70% ethanol, or 0.5% Oxycide Plus™. Materials used to wipe up spills may require biodegradation considerations.
6. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous waste must be handled and discarded in accordance with all local, regional, and national regulations.
7. The Aspergillus GM Lateral Flow Test Strips (REF LFAF50) may be biohazardous after running specimens. Handle and dispose of accordingly.
8. Refer to Hazards and Precautionary Information section for hazards associated with specific reagents. Safety Data Sheets are available upon request.
9. Results read after the 10-minute reading window is invalid.

For Interpretation using Results of LFA

1. Screw the lid on tightly and vortex the sample.
2. Place tube in a heat block for 5-6 minutes at 22°C.
3. After pre-treatment, treated sample can be stored at 2-4°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 (REF AFAGMP) into one clean tube or microwell and add 120 µL of Aspergillus GM LFA Running Buffer (REF AFRLBF) 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 (REF AFRLBF) 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 into 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.

READ 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

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”. The sōna Aspergillus GM LFA Cube Reader comes with an RFID tag. Scan the code tag on the bottom of the Aspergillus GM Lateral Flow Test Strip Tube (REF AFULATF50) 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.
3. When the test strip is ready to be analyzed, properly insert the LFA strip into cube reader on the sample arrows of the strip are facing the same direction as the sample arrows on the adapter itself. Results should be read within 10 minutes of completion of the test’s 30-minute incubation.
4. 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.
5. 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. The results displayed in the Cube Reader are the same value as the results displayed on the Screen Display.
6. 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.

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 occurred. A positive control (Aspergillus GM Positive Control 1) can be evaluated by adding 120 µl in 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 occur when no control line is visible or when the control line intensity is not maintained throughout the boil during the pretreatment.

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 pretreated 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 nose/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 RAMY Customer Service for assistance and for further information.

CROSS-REACTIVITY ANALYSIS
The sīna 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.

<table>
<thead>
<tr>
<th>Pathology</th>
<th># of Samples</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopus</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Sphingodium</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Rhizoctonia</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Mucor</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Penicillium</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CMV</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>RV</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Rheumatococcus</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>5</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>5</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Transplant</td>
<td>5</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

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

**SPECIFIC PERFORMANCE CHARACTERISTICS**

The sīna 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.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Point Estimate</td>
<td>97%</td>
<td>100%</td>
<td>97%</td>
<td>100%</td>
</tr>
<tr>
<td>95% CI 85-100%</td>
<td>92-99%</td>
<td>72-83%</td>
<td>77-87%</td>
<td></td>
</tr>
</tbody>
</table>

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

**REPRODUCIBILITY AND PRECISION**
The sīna AGM LFA was evaluated for reproducibility and precision by spiking serum and artificial BAL (aBALs) 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.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Neg</td>
<td>63</td>
<td>84%</td>
<td>73</td>
<td>99%</td>
<td></td>
</tr>
<tr>
<td>Low Pos</td>
<td>48</td>
<td>64%</td>
<td>52</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td>Med Pos</td>
<td>75</td>
<td>100%</td>
<td>74</td>
<td>99%</td>
<td></td>
</tr>
</tbody>
</table>

**CLEANING THE CUBE READER**
1. Remove the sīna LFA Cube Reader from the adapter by gently applying downwards pressure on the adapter tab and lifting the cube reader out of adapter.
2. Clean the LFA Cube Reader Adapter with a disinfectant. See Warnings for Users.
3. Clean the cube reader lens with a lint-free cloth.
4. 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**
- APPF01, ALFIRA1: Contain Boric Acid
- Signal word: Danger

**Precautionary Statement(s)**

P410 May damage fertility or the unborn child.

**Precautionary Statement(s)**

P280 Do not handle until all safety precautions have been read and understood.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P330 If exposed or concerned. Get medical advice/attention.

P303 Store locked up.

P301 Dispose of contents/container in accordance with local regulations.

**BIBLIOGRAPHY**