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 fungi 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 42% 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 LFAF50) is added, followed by an LFA strip (REF LFAFS0). 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 sample specimen 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. In Vitro Diagnostic Use only.
2. 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.

REAGENTS

1. Aspergillus GM Lateral Flow Test Strips (50 each) (REF LFAPA0) — LFA dipstick packaged into a desiccated container with a cap.
2. Sample Pretreatment Buffer (7 mL) (REF AFSFB1) — EDTA solution containing a preservative.
3. Aspergillus GM Positive Control (3 mL) (REF AFPSO1) — Aspergillus Galactomannan in a saline solution containing a preservative.
4. Aspergillus GM LFA Running Buffer (3 mL) (REF AFRLB) — LFA running buffer containing a preservative.
5. Package Insert.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable gloves.
2. Protective glasses.
3. Pipettes(s) capable of measuring and delivering up to 300 µL and associated disposable tips.
4. 1.2-5.0 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.

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 IF 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 pathogenic 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 AFSFB1) 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 (v/v/ 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 APCS01) into a clean tube or microwell and 120 µL of AGM LFA Running Buffer (REF AFRLB) into a clean tube or microwell. It is recommended that controls be tested monthly.
2. Note: Do not boil negative and/or positive controls.
3. Pipette 80 µL of supernatant from treated serum/BAL into a separate clean tube or microwell. Place one AGM Lateral Flow Strip (REF LFAPA0) test strip in each tube or microwell from step 2.
4. Place one AGM Lateral Flow Strip (REF LFAPA0) 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.

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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**

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 present, test conditions may be subject to variation. 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 supports 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/antibiotics used to num the neck/throat area for the aspiration process has not been evaluated.

**CROSS-REACTIVITY ANALYSIS**

The sôna 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.

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 Paracoccidioides brasiliensis, Coccidiodes, Histoplasma, and Candida exhibited some cross-reactivity. Antigens from the following organisms were tested and exhibited no cross-reactivity:

- Blastosomyces
- Cryptococcus

**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 sôna 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.

**BIBLIOGRAPHY**


**REPRODUCIBILITY AND PRECISION**

The sôna AGM Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum and artificial BAL (sBAT) with Aspergillus GM antigen to produce a panel consisting of a negative sample, a high-negative (CJ) sample, a low-positive sample, a moderate-positive sample and a high positive (CJ). This panel was tested in triplicate, daily, for 5 days at one site. The results of this study are shown in the tables below.