IVD REF ASP-025

FUNGADIA

Aspergillus Antigen

Instruction for Use – English

Please read this user manual carefully before using the test

INTENDED USE

FungaDia-Aspergillus is a rapid immunochromatographic test for the qualitative detection of the Aspergillus galactomannan antigen in serum and bronchoalveolar lavage (BAL) fluid from adult or pediatric patients suspected of Fungal infections. The detection of galactomannan in serum or BAL can be used as an aid in the diagnosis of invasive aspergillosis (IA). This kit is for medical professional use only and should be used in combination with other diagnostic techniques such as microbiological culture, histological examination of biopsies or radiological examination.

SUMMARY

Aspergillosis is a term for infections caused by fungi belonging to the genus Aspergillus, whose spores are airborne and inhaled by all individuals. The species *Aspergillus fumigatus* is responsible for over 80% of human aspergillosis. Invasive aspergillosis affects the lower respiratory tract after inhalation of these spores. It occurs mainly in neutropenic patients (anti-cancer treatment), in patients treated with immunosuppressants and corticosteroids (following bone marrow transplantation) and in patients hospitalized in intensive care for severe respiratory disease (influenza, COVID-19). Symptoms include fever, cough, chest pain, hemoptysis, and breathing difficulties. Due to the lack of typical clinical manifestations and effective early diagnostic methods, IA can have a mortality rate of up to 50%. Rapid and early detection is a key factor in the effective treatment and reduction of mortality of IA.

DETECTION PRINCIPLE

The principle of the test is colloidal gold immunochromatography. If the sample is positive, the antigens in the sample react with the red-colored nanoparticles and form a complex (Antigen - anti-Aspergillus monoclonal antibodies – gold nanoparticles), which was previously pre-dried on the conjugate pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-Aspergillus antibodies present on the membrane (Test line) capture the colored conjugate complex and a red line will appear. If the sample is negative, there is no Aspergillus antigens present or the antigens may be present in a concentration lower than the detection limit. The anti-Aspergillus antibodies present on the membrane (Test line) will not capture the antigen-red-colored conjugate complex (not formed), and the red line will not appear. Whether the sample is positive or not, the nanoparticle complex continues to move across the membrane to the immobilized specific antibodies placed in the control line. The anti-mouse antibodies present on the membrane will react with the anti-Aspergillus antibodies coated on the gold nanoparticles and capture the complex to form a red line. The presence of this control red line serves as: (1) verification that sufficient volume is added, (2) that proper flow is obtained and (3) an internal control for the reagents. The control line must always appear.

KIT COMPONENTS

Components	Quantity per kit
Aspergillus Galactomannan Detection Test	25
Positive Control (50 ng/ml Galactomannan)	1 x 1.0 mL
Negative Control	1 x 1.0 mL
Sample treatment solution	1 x 3.0 mL
Instructions for use	1

Note: The components of kits cannot be exchanged

Materials Required but Not Supplied

1. Pipettes and sterile tips
2. Timer
3. Disposable sterile micro-centrifuge tubes (eg. 72.692.005, Sarstedt)
4. Centrifuge
5. Heat incubator

STORAGE CONDITIONS AND SHELF LIFE

1. Store at 2-30°C for 24 months, store in a dry and cool place.
2. The rapid test should be used within 1 hour after opening the aluminum foil bag. The reagent in the bottle can be stored at 2-30°C.
3. The date of expiration is printed on the label.

SAMPLE PREPARATION

1. Sample type: Serum and BAL fluid
2. Specimen collection: Collect patient sample according to the clinical collection guidelines for laboratory test samples;
3. Avoid contamination during sample collection, transportation, and preservation.
4. The sample should be stored at 2-8 °C for 48h (Serum samples) and 24 h (BAL samples) if the samples cannot be tested in time, store below -20°C. Store treated samples below -20°C, 6 months.
5. Avoid sample contamination, deterioration and repeated freeze-thaw.
6. Grossly hemolyzed, icteric or lipidemic specimens are not recommended for testing.

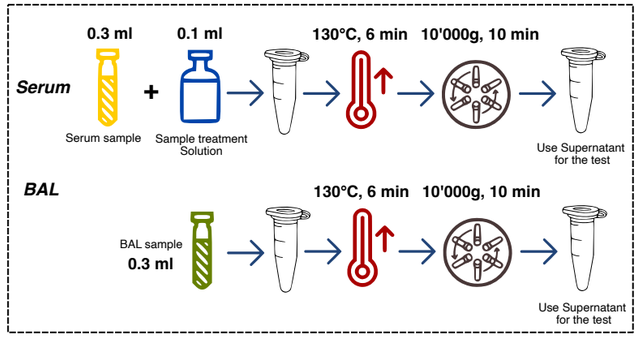
TEST PROCEDURE

1. Sample Pre-treatment

Serum: Take 300 µl of serum into a centrifuge tube, add 100 µl of sample treatment solution and mix thoroughly;

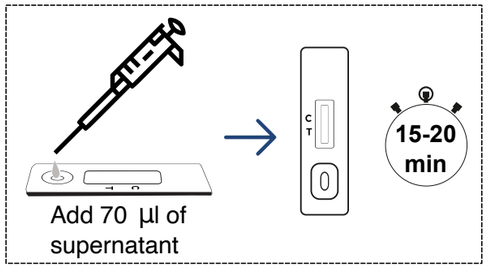
BAL fluid: Take 300 µl of BAL fluid into a centrifuge tube, the sample treatment solution is not required for the BAL fluid.

- Put the centrifuge tube in a water/metal bath at 130 °C for 6 minutes (or 100°C for 7 min);
- Take the centrifuge tube out of the water/metal bath and centrifuge at 10'000 x g for 10 min;
- Use the supernatant for testing



2. Detection

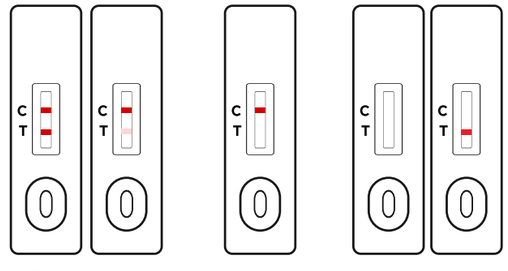
- Take out the test from aluminum foil bag and place it on the clean horizontal bench;
- Add **70 µl** of supernatant to the sample well (S) of the test device;
- Incubate for **15-20 minutes** and read the result. Do not move the test during incubation. Do not interpret the result **after 30 minutes.**



RESULTS INTERPRETATION

The presence of two lines (line T and line C), **regardless of the intensity of the test line**, indicates a positive result. If the control line (line C) does not appear, the result is invalid and the test should be repeated.

1. Negative results cannot exclude Aspergillus infection, and it is possible that the sample is collected before the appearance of Aspergillus Galactomannan antigens or the concentration is below the limit of detection (LOD).
2. The color intensity of the test results cannot be used as the basis for determining the total content of Aspergillus antigen.



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QUALITY CONTROL

1. Positive control: 70 µl of non-treated positive control is used for detection directly, the test result must be positive (T line positive).
2. Negative control: 70 µl of non-treated negative control is used for detection directly; the test result must be negative (no T line).

LIMITATIONS

1. The product is only used for the detection of Aspergillus Galactomannan antigen in serum and BAL samples.
2. The test results of this kit are for reference only and should not be used as the only basis for clinical diagnosis and treatment. The clinical management of patients should be comprehensively considered in conjunction with their symptoms, medical history, other laboratory tests and treatment responses.

PERFORMANCES

1. Limit of detection (LOD)

The limit of detection (LOD) has been evaluated with 3 different lots and is 2 ng/ml of Galactomannan antigen.

2. Hook effect

Very high concentrations of Aspergillus hyphal antigen could cause a hook effect (> 0.2 mg/ml). It is recommended to use a physiological saline solution to make a 5-10 times dilution of the sample.

3. Interfering substances and cross-reactions

There are no known interfering substances and cross-reactive diseases. 50 ng/ml of (1-3)-β-D-glucan, Candida mannan and Cryptococcal capsular polysaccharides, as well as cross-reactive diseases, were tested. Food supplements containing galactomannan, maltodextrin or corn starch (Abbott Nutrition) were tested and no interference was observed at a concentration of 0.5%.

Disease states	Aspergillus positive (ELISA)	Aspergillus negative (ELISA)	Cross-reactivity
Bacterial Sepsis	0	9/9	NO
COVID-19 positive	0	3/3	NO
HBsAg	0	5/5	NO
H.pylori IgG	0	3/3	NO
Anti-CCP	0	3/3	NO
F.rhumatoides IgG	0	3/3	NO
Anti-ANA antibody	0	3/3	NO
ANA positive	0	2/2	NO

POSITIVE SAMPLES

Potential interfering substances	Concentration	Galactomannan	Results
(1,3)-β-D-glucan	50 ng/ml	6 ng/ml	POS (3/3)
Candida albicans mannan	50 ng/ml		POS (3/3)
Cryptococcal capsular polysaccharide	50 ng/ml		POS (3/3)

NEGATIVE SAMPLES

Potential interfering substances	Concentration	Results
(1,3)-β-D-glucan	50 ng/ml	NEG (3/3)
Candida albicans mannan	50 ng/ml	NEG (3/3)
Cryptococcal capsular polysaccharide	50 ng/ml	NEG (3/3)
Galactomannan from Carob	1 mg/ml	NEG (3/3)
Amoxicilline (Sandoz)	0.75 mg/ml	NEG (3/3)
Microorganisms		
Aspergillus fumigatus ATCC 204305	10^7 cfu/ml	POS (3/3)
Aspergillus fumigatus BEI NR-41311	10^7 cfu/ml	POS (3/3)
Aspergillus fumigatus BEI NR-35301	10^7 cfu/ml	POS (3/3)
Aspergillus fumigatus BEI NR-35302	10^7 cfu/ml	POS (3/3)
Aspergillus fumigatus BEI NR-35303	10^7 cfu/ml	POS (3/3)
Aspergillus fumigatus BEI NR-41312	10^7 cfu/ml	POS (3/3)
Aspergillus niger ATCC 16888	10^7 cfu/ml	POS (3/3)
Aspergillus flavus ATCC 9643	10^7 cfu/ml	POS (3/3)
Aspergillus oryzae ATCC 10124	10^7 cfu/ml	POS (3/3)
Aspergillus brasiliensis ATCC 9642	10^7 cfu/ml	POS (3/3)
Aspergillus ustus ATCC 10760	10^7 cfu/ml	POS (3/3)
Aspergillus caesiellus ATCC 42693	10^7 cfu/ml	POS (3/3)
Aspergillus terreus Thom ATCC 1012	10^7 cfu/ml	POS (3/3)
Aspergillus nidulans ATCC 10074	10^7 cfu/ml	POS (3/3)
Penicillium chrysogenum ATCC 10106	10^7 cfu/ml	POS (3/3)
Penicillim digitatum ATCC 48113	10^7 cfu/ml	POS (3/3)
Paecilomyces variotii ATCC 18502	10^7 cfu/ml	POS (3/3)
Talaromyces (Penicillium) marneffei	10^7 cfu/ml	NEG (3/3)
Cladosporium cladosporiodes ATCC 16022	10^7 cfu/ml	NEG (3/3)
Magnusiomyces capitatus ATCC 28576	10^7 cfu/ml	NEG (3/3)
Alternaria alternata ATCC 66981	10^7 cfu/ml	NEG (3/3)
Lishtheimia ramose ATCC 22754	10^7 cfu/ml	NEG (3/3)

4. Repeatability and reproducibility

Reproducibility and reproducibility of the test have been evaluated internally with three different lots and a coefficient of variation (CV) of less than 10% was observed.

5. Clinical performances

A total of 153 serum and 34 Bronchoalveolar lavage samples were used to conduct a retrospective clinical evaluation in one university hospital in France. The reference method was the CE-Marked PLATELIA™ ELISA Aspergillus Ag Galactomannan assay (BioRad, Marne-la-Coquette, France). Further prospective and retrospective clinical evaluations are ongoing in 2 university hospitals in France. The first study was conducted on 84 samples, with a sensitivity of 85.7% and a specificity of 89.3%.

Serum+LBA	Platelia	
FungaDia RDT	+	-
+	40	5
-	11	127
Sensitivity:	**78,4%**	(CI95%: 64,3-88.2%)
Specificity:	**96,2%**	(CI95%: 90,9-98.6%)
PPV:	**88,9%**	(CI95%: 75,1-95,8%)
NPV:	**92,0%**	(CI95%: 85,9-95,8%)

Serum+LBA	Platelia	
FungaDia RTD	+	-
+	48	3
-	8	25
Sensitivity:	**85,7%**	(CI95%: 73,2-93.2%)
Specificity:	**89,3%**	(CI95%: 70,6-97.2%)
PPV:	**94,1%**	(CI95%: 82,8-98.5%)
NPV:	**75,8%**	(CI95%: 57,4-88.3%)

WARNING AND PRECAUTIONS

1. This product is used for in vitro diagnosis, professional use only.
2. Do not reuse the test. Do not use the test after expiry date
3. Please read the test results within the specific time to avoid wrong interpretation.
4. Do not use the components from different batches or different types of reagents.
5. Properly dispose the specimen and used materials following the local biohazardous disposal regulation.
6. Use protective equipment when handling samples and tests as they may contain infectious agents and human or animal components.
7. Sodium azide is used as preservative in the sample treatment solution. Dispose material according to relevant local regulations and avoid contact with eyes and skin.
8. When the content of Aspergillus antigen in the sample is very high, the line C may be weakened.
9. Very high concentrations of Aspergillus antigen cause a hook-like effect, leading to false negative results. In this case, it is recommended to use a physiological saline solution to make a 5-10 times dilution of the sample.
10. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

REFERENCES

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3. White PL. et al. Evaluation of real-time PCR, galactomannan enzyme-linked immunosorbent assay (ELISA), and a novel lateral-flow device for diagnosis of invasive aspergillosis. J Clin Microbiol. 2013; 51(5):1510–6.
4. Hoenigl M. et al. Performance of galactomannan, beta-D-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol. 2014a; 52(6):2039–45.
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6. Johnson GL. et al. Aspergillus-specific lateral-flow device and real-time PCR testing of bronchoalveolar lavage fluid: a combination biomarker approach for clinical diagnosis of invasive pulmonary aspergillosis. J Clin Microbiol. 2015; 53(7):2103–8.

SYMBOLS

	Manufacturer		Expiry Date
	Do not reuse	LOT	Lot Number
	Manufacturing date	EC REP	European Authorized Representative
	Consult instructions for use	IVD	In vitro diagnostic medical device
	Temperature limitation	REF	Catalog number
	Sufficient for <n> Test	CE	CE Marking
	Not for near patient testing (IVDR only)		Not for self-testing (IVDR only)

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CE IVD SWISS QUALITY

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