IVD REF ASPE-096

FUNGADIA

Aspergillus Antigen ELISA

Instruction for Use – English

Please read this user manual carefully before using the test

INTENDED USE

FungaDia-Aspergillus Antigen ELISA is an enzyme sandwich microplate immunoassay Kit for the qualitative detection of Aspergillus galactomannan antigen in adult and pediatric serum samples and bronchoalveolar lavage (BAL) fluid samples of patients with symptoms of, or medical conditions predisposing the patient to, invasive Aspergillus infection in clinical laboratories. The detection of galactomannan in serum or BAL can be used as an aid in the diagnosis of invasive aspergillosis (IA). This ELISA kit should be used in combination with other diagnostic techniques such as microbiological culture, histological examination of biopsies or chest X-ray 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. The detection of galactomannan in serum or BAL is a diagnostic method recommended by recent guidelines [4].

DETECTION PRINCIPLE

FungaDia Aspergillus Galactomannan ELISA Detection Kit is a one-step enzyme sandwich microplate immunoassay which detects galactomannan in human serum and BAL fluid. The assay uses specific mouse monoclonal antibody against Aspergillus galactomannan. The monoclonal antibody is used to coat the wells of the microplate and bind the antigen (capture antibody) and to detect the antigen bound to the sensitized microplate (conjugate reagent: peroxidase-linked monoclonal antibody). Serum or BAL fluid samples are heat-treated in the presence of EDTA to dissociate immune complexes and precipitate proteins that could possibly interfere with the test. The treated samples and conjugate are added to the wells coated with monoclonal antibodies and incubated. A monoclonal antibody - galactomannan - monoclonal antibody/peroxidase complex is formed in the presence of galactomannan antigen. The strips are washed to remove any unbound material. Then, the Chromogen TMB solution is added, which will react with the complexes bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of an acidic solution, which changes the blue color to yellow. The absorbance (optical density) of specimens and controls is determined with a spectrophotometer at 450 nm.

KIT COMPONENTS

Quantity	Label	Name	Contents
1	R1	ELISA Microplate	12x8 Microwell plate coated with anti-galactomannan antibody
1 x 25 ml	R2	Concentrated Washing Solution (20x)	PBST Buffer
1 x 2 ml	R3	Negative Control	PBST Buffer
1 x 2 ml	R4	Cut-off Control	PBST Buffer, galactomannan
1 x 2 ml	R5	Positive Control	PBST Buffer, galactomannan
1 x 7 ml	R6	Antibody conjugated with HRP	Anti-galactomannan antibody conjugated with HRP
1 x 12 ml	R7	Sample Treatment Solution	EDTA solution
1 x 25 ml	R8	Chromogen TMB Solution	Tetramethyl benzidine (TMB)
1 x 12 ml	R9	Stop Solution	H_2SO_4
5	M1	Plate Sealer	Microwell plate adhesive film
1	IFU	Instruction for use	

Note: Components in different batch kits are not interchangeable

TMB (3,3',5,5'-tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase.

STORAGE CONDITIONS AND SHELF LIFE

The Aspergillus Galactomannan ELISA Detection Kit can be stored at 2-8°C until the expiry date printed on the label. Store opened buffer bottles and the unused microwells in the original pouch, with desiccant and correctly closed at 2-8°C for 6 months. The transport of the kit can be done at room temperature (5-30°C).

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized water, for dilution of Concentrated Washing Solution (**R2**)
2. Absorbent paper
3. Protective equipment (disposable gloves and protective glasses)
4. Pipettes or multi-channel pipettes
5. Screw cap micro tubes 1.5 ml (recommended: 72.692.005, Sarstedt)
6. Dry Block Incubator 130°C
7. Centrifuge for 1.5 mL polypropylene tubes (10,000 *xg*)
8. Vortex agitator
9. Microplate incubator at 37 ±1°C
10. Microplate reader equipped with 450 nm filters

Note: Automated ELISA Processor (eg. Evolis, BioRad) can be used.

SAMPLES REQUIREMENTS

Sample type: Serum, BAL fluid

Collect patient sample according to the clinical collection guidelines for laboratory test samples. Avoid contamination during sample collection, transportation, and storage.

The sample should be stored at 2-8 °C. Serum samples should not exceed 48h, if cannot be tested within 48h, please store at -20°C; BAL fluid should not exceed 24h, if cannot be tested within 24h, please store at -20°C. Samples are stable 1 month at -20°C. Treated samples can be stored for 6 months at -20°C. Avoid sample contamination and repeated freeze-thaw cycles. Grossly hemolyzed, icteric or lipidemic specimens should be avoided.

TEST PROCEDURE

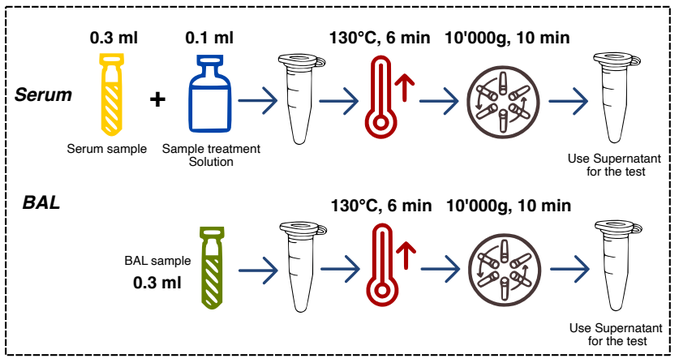
1. Sample Treatment and preparation

Serum:

1. Pipette 300 µL of sample into individual polypropylene tube.
2. Add 100 µL Sample Treatment Solution (**R7**) to each tube.
3. Mix tubes thoroughly by vortexing.
4. Incubate for 6 minutes at 130°C (or 7 minutes at 100°C).
5. Centrifuge at 10000 *xg* for 10 min.
6. Use 50 µL of supernatant for detection.

BAL Fluid:

1. Pipette 300 µL of sample into individual polypropylene tube.
2. Incubate for 6 minutes at 130°C (or 7 minutes at 100°C).
3. Centrifuge at 10000 *xg* for 10 min.
4. Use 50 µL of supernatant for detection.



2. Enzyme Immunoassay Procedure

1. Bring all reagents to room temperature (18-25°C).
2. Open the plate aluminum pouch and take out the microwell plate (**R1**).
3. Prepare the Working Washing Solution by diluting the Concentrated Washing Solution (20×) (**R2**) 1/20 with sterile distilled water or ultra-pure water.
4. Add 50 µL of Anti-galactomannan Antibody Conjugated with HRP (**R6**) to each well.
5. Add 50 of µL samples or controls per well:
   a. Controls (non-treated): Use one well for the Negative Control (**R3**), two wells for the Cut-off Control (**R4**), and one well for the Positive Control (**R5**).
   b. Samples (treated): 50 µL of treated supernatant to each well.

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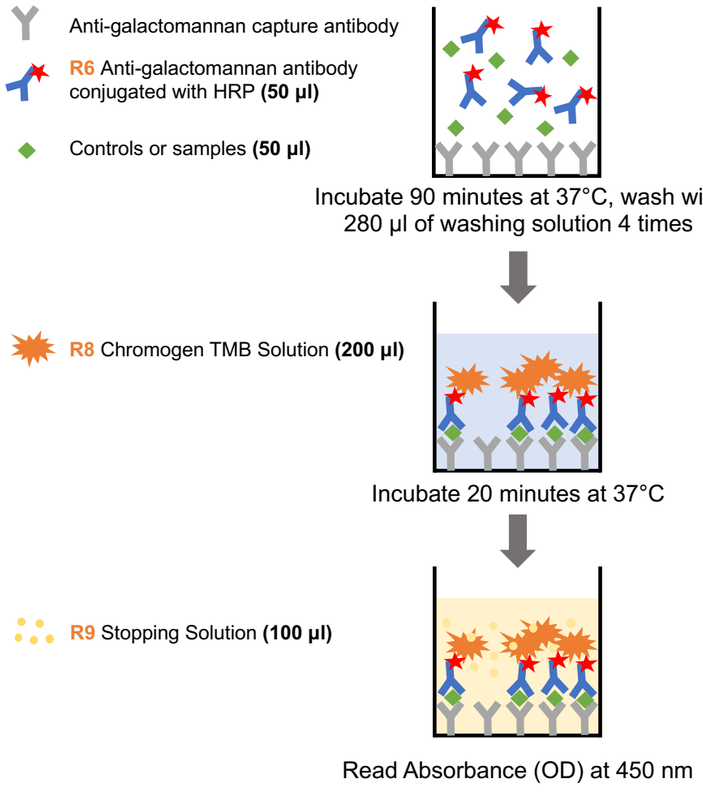
For assays of more than 25 samples, we recommend filling the three first and the three last wells with the controls as a duplicate.

6. Cover plate with plate sealer and incubate at 37°C (±1°C) for 90 ± 5 min.
7. Remove the contents of all wells into a waste container. Invert the microwell plate and gently tap on absorbent paper to remove the remaining liquid. Wash 4 times the wells with 280 µL of Working Washing Solution.
8. Rapidly add 200 µL of the Chromogen TMB Solution (**R8**) to each well, avoid exposure to light. Incubate in the dark for 20 min at 37°C (without plate sealer).
9. Stop the reaction by adding 100 µL of Stopping Solution (**R9**) to each well. Mix well by gently shake, if needed, wipe the bottom of the wells and eliminate bubbles, and then read the value at 450 nm within 1 hour.



QUALITY CONTROL

Control	Calculation	Specification
Negative	Negative Control OD / Mean Cut-off Control OD	Negative Control Ratio **< 0.4**
Cut-off	Mean absorbance Value (A)	Absorbance (A) of cut-off **> 0.2**
Positive	Positive Control OD / Mean Cut-off Control OD	Positive Control Ratio **> 1.5**

The test is valid only if these previous criteria are met.

RESULTS INTERPRETATION

For result interpretation, the Sample Index (SI) is determined as follow:

$$Sample\ Index\ (SI) = \frac{Absorbance\ Sample}{Mean\ Absorbance\ of\ Cut-off}$$

Reference range for serum and BAL is determined as follow:

Sample Index (SI)	Results interpretation
< 0.5	Considered to be **negative** for galactomannan antigen and indicate that the risk of invasive Aspergillus infection is low.
≥ 0.5	Considered to be **positive** for galactomannan antigen and indicate that the risk of invasive Aspergillus infection is high.
A grey zone could be determined by each lab based on the tested population. If the result is close to the cut-off index (SI: 0.4-0.5) or doubtful, we recommend to retest with a new sample.	

LIMITATIONS

1. Read the package insert carefully before performing the test. Procedures and the Interpretation of Results must be followed carefully.
2. A negative test from serum and/or BAL samples cannot exclude the diagnosis of Invasive Aspergillosis. Samples from patients at risk for invasive aspergillosis should be tested twice a week or with other diagnostic procedures.
3. A positive result with no clinical signs could be due to the early galactomannan antigen detection in serum or BAL, before the appearance of clinical and/or radiological signs.
4. Cross-Contamination of negative patient specimen wells by positive control/patient specimen wells is possible if the contents of one well spill over into another well due to rough handling of the microplate or poor pipetting technique.
5. FungaDia Aspergillus Galactomannan ELISA Detection Kit has not been evaluated for use with plasma or other sample types such as urine or CSF.
6. The results of FungaDia Aspergillus Galactomannan ELISA Detection Kit in Bronchoalveolar Lavage (BAL) samples from non-immunocompromised or neonatal patients should be interpreted with caution.
7. Samples with results close to cut-off index (SI: 0.5) must be interpreted carefully. The clinical management of patients and diagnosis of infectious diseases should be comprehensively considered in conjunction with their symptoms, medical history, other laboratory tests and treatment responses.

PERFORMANCES CHARACTERISTICS

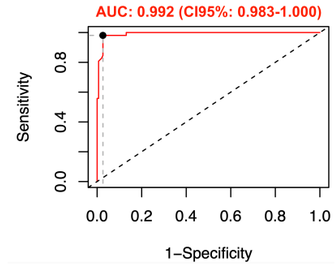
CLINICAL PERFORMANCE

A retrospective clinical study on 205 serum and 33 BAL samples was conducted in a University Hospital in France. FungaDia-Aspergillus ELISA performance was compared with the reference Aspergillus Ag ELISA Assay Platelia™ (BioRad, Marnes-la-Coquette, France) [8].

Serum Samples

Platelia (BioRad)/Clinical diagnostic

		+	-
FungaDia ELISA	+	49	1
	-	1	152
Sensitivity:	98,0%	(CI95%: 87.8-100%)	
Specificity:	99,3%	(CI95%: 95.9-100%)	
PPV:	98,0%	(CI95%: 88.4-99.9%)	
NPV:	99,3%	(CI95%: 95.9-100%)	

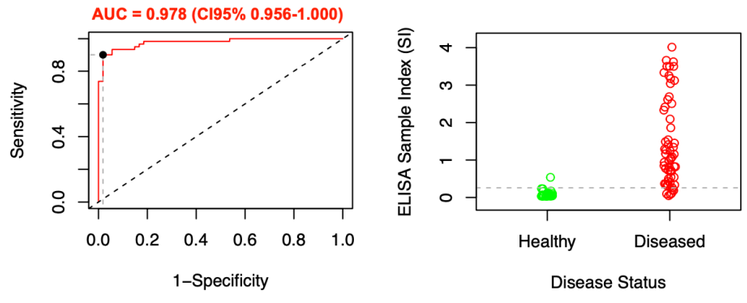


BAL Samples

Platelia (BioRad)

		+	-
FungaDia ELISA	+	5	0
	-	0	28
Sensitivity:	100,0%	(CI95%: 46.3-100%)	
Specificity:	100,0%	(CI95%: 85.0-100%)	
PPV:	100,0%	(CI95%: 46.3-100%)	
NPV:	100,0%	(CI95%: 85.0-100%)	

A second clinical evaluation on 115 samples (48 retrospective and 67 prospective samples) was conducted in a University Hospital Center in France. The performance of FungaDia-Aspergillus ELISA kit was compared to the reference test Aspergillus Ag ELISA Assay Platelia™ (BioRad, Marnes-la-Coquette, France). A sensitivity of 90.2% and a specificity of 98.1% was determined. The ROC curve had an area under the curve (AUC) of 0.978.



CROSS-REACTIVITY

Cross-reactivity of BAL fluid samples with *Mycoplasma pneumoniae* or anesthetic drugs used for the aspiration process has not been evaluated. Other fungi presented in the table below, have shown reactivity with monoclonal antibody used in the assay.

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)

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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)
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)
Trichophyton interdigitale ATCC 9533	10^7 cfu/ml	NEG (3/3)
Trichophyton rubrum ATCC 28188	10^7 cfu/ml	NEG (3/3)
Candida albicans	10^7 cfu/ml	NEG (3/3)
Candida parapsilosis	10^7 cfu/ml	NEG (3/3)
Candida glabrata	10^7 cfu/ml	NEG (3/3)
Candida tropicalis	10^7 cfu/ml	NEG (3/3)
Candida krusei	10^7 cfu/ml	NEG (3/3)
Coccidioides immitis	10^7 cfu/ml	NEG (3/3)
Histoplasma capsulatum	10^7 cfu/ml	POS (3/3)

INTERFERRING SUBSTANCES

Interference with Galactomannan from carob bean gum was observed at a concentration of 1 mg/ml. No interferences with Amoxicilline (Sandoz) up to 7.5 mg/ml and with Gamma globulins have been observed. Interference with food supplements containing Galactomannan, maltodextrin or hydrolyzed corn starch was observed using 1% solution of Prosure® and Enlive® (Abbott Nutrition). Positive reactions in the absence of clinical signs may be observed in patients receiving other products containing galactomannan or antibiotics (Piperacillin...).

LIMIT OF DETECTION

The limit of detection (LOD) has been determined at 0.5 ng/ml of Aspergillus Galactomannan and the limit of quantification (LOQ) at 1.0 ng/ml.

PRECISION

Repeatability was assessed by testing positive samples in 10 wells on 1 assay. The coefficient of variation (CV) was 5.6%. The batch-to-batch reproducibility was assessed by testing positive samples 10 times on 3 different lots, the average coefficient of variation (CV) was 8.9%.

WARNING AND PRECAUTIONS

1. For *in vitro diagnostic* use only. For professional use only, not for self-testing nor near-patient testing.
2. Store frozen serum or BAL fluid samples properly to avoid contamination or degradation of samples.
3. Do not use kit or kit reagents after expiry date.
4. Do not mix reagents with different lot numbers.
5. Do not re-use microwells. Disassemble the microwell from the plate support with care to not break the microwells.
6. Bring all reagents to room temperature for at least 30 minutes before use.
7. Avoid the formation of bubbles in the wells and mix reagents thoroughly before use
8. Mix thoroughly the Concentrated Washing Solution (**R2**) before preparing the Working Washing Solution. Crystallization of concentrated washing solution is possible, rinse well the bottle.
9. Use separate and clean pipettes tips for each sample
10. Comply with the recommended number of wash cycles and ensure that all wells are completely filled and then completely emptied.
11. Do not allow the microwells to dry between the end of the wash cycle and addition of reagents.
12. Do not put the Conjugate and Chromogen TMB Solution in the same container.
13. Do not allow Conjugate or Chromogen TMB Solution to come into contact with metal or metallic ions and avoid exposure to strong light
14. Stopping Solution contains acid, avoid contact with eyes and skin.
15. Use protective equipment when using the test and handling samples as they may contain infectious agents, human or animal components.
16. All materials used for this test could contain hazardous substances and human or animal origin components. Refer to national and regional laws and regulations for the disposal of hazardous waste.
17. Keep testing materials (tubes, tips, containers, etc.) clean, dust-free and sterile to minimize contamination with Aspergillus spores from the environment.
18. The Chromogen TMB Solution must be colorless. The appearance of a blue color indicates the reagent is contaminated and should not be used.
19. 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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6. Jenks et al, Invasive aspergillosis in critically ill patients: Review of definitions and diagnostic approaches, Mycoses. 64(9):1002-1014 (2021)
7. Fekkar et al., Occurrence of Invasive Pulmonary Fungal Infections in Patients with Severe COVID-19 Admitted to the ICU, Am J Respir Crit Care Med. 203(3):307-317 (2021)
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SYMBOLS

	Manufacturer		Expiry Date
	Do not reuse	LOT	Lot Number
EC REP	European Authorized Representative	CONTROL ±	Cut-off Control
CONTROL -	Negative Control	CONTROL +	Positive Control
	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		Not for self-testing

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SWISS QUALITY

CE
IVD

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