Human Aspergillus fumigatus IgG Antibody ELISA Kit

Enzyme Immunoassay for the determination of Aspergillus fumigatus IgG in serum and plasma

Catalog number: ARG80513

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Aspergillus species of known pathogenicity to man are Aspergillus fumigatus, A. flavus, A. niger, A. terreus and A. nidulans. The most common pathogen of this genus is A. fumingatus which occurs in hay, grain, rotten plants and birds' faeces. The main opportunistic invasive fungal infections are the candidal mycosis followed by aspergillosis. Generally infections with Aspergillus spp. are airborne.

Because of the ubiquity of Aspergillus species it renders more difficult to decide between contamination by commensals or a serious infection. Usually infection in man occurs in already damaged tissues only. *Aspergillus spp.* can cause a chronical infection of paranasal sinus, eyes or lungs.

Three types of lung-aspergillosis can be distinguished:

a: Acute infection (bronchial pneumonia; pneumonia)

Aspergillus pneumonia is mostly found in patients with neutropenia (decrease of neutrophil granulocytes), after a long-time therapy with glucocorticoids, in immunosuppressed patients (after organ transplantation) and in alcoholics.

b: Saprophytic aspergillom (compact reticulum of hyphae in the lungs)

Preformed caves in the lung due to a previous tuberculosis give place to a colonisation of Aspergillus species.

c: Allergic bronchopulmonary aspergillosis (ABPA)

This clinical symptoms is due to a hypersensitive reaction of the bronchial system (mediated by IgE) after inhalation of aspergillus spores. Subsequently

the bronchial system produces highly viscous secretions that may block the bronchial lumen. The patient develops difficulties of breathing and a fibrosis.

Next to ELISA the indirect Aspergillus hemagglutination test (Aspergillus HAT) can be performed to detect specific IgG and IgM antibodies. The HAT is not suitable as a screening test, however, because of its low sensitivity. In some high-risk patients it shows only low antibody titers. For a better diagnosis of invasive aspergillosis the brain or lung of these patients should be examined by a biopsy.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A specific Aspergillus antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Aspergillus antibody present is bound by the immobilized antigen. After washing away any unbound substances, an HRP-conjugated antibody specific for human IgG is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Aspergillus IgG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A (Negative Control)	2ml	4°C
Calibrator B (Cut-off Standard)	2ml	4°C
Calibrator C (Weak Positive Control)	2ml	4°C
Calibrator D (Positive Control)	2ml	4°C
HRP-conjugated antibody	15ml (Ready-to-use)	4°C
Sample Diluent	60ml	4°C
10X Wash buffer	60ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

For the performance of the test, the samples have to be diluted 1:101 with sample diluent.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls, diluted samples (1:101) and zero controls (sample diluent buffer) into wells. Incubate for 1h at RT.
3. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl HRP-conjugated antibody (ready-to-use) into each well. Cover wells and incubate for 30 minutes at RT.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of TMB Reagent to each well. Incubate for 20 minutes at room temperature.
7. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

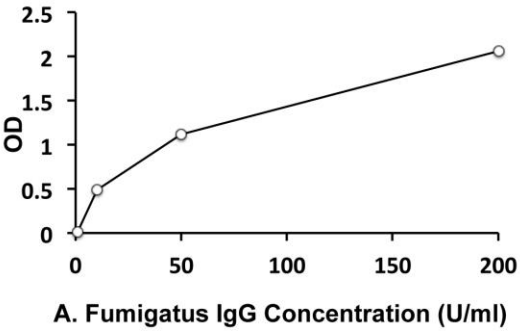
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



INTERPRETATION OF RESULTS

<8 U/ml (negative); 8-12 U/ml (Equivocal); >12 U/ml (Positive)

QUALITY ASSURANCE

Sensitivity

The mean MDD was 1.08 U/ml.

Assay Range: 1-200 U/ml

Specificity

No cross reactivity was observed with the following factors:

Candida albicans

Intra-assay and Inter-assay precision

The CV value of intra-assay precision is 9.9% and inter-assay precision is 11.1%.

Recovery

87-97%

Inter-lot Precision

3.5-16.4%

Linearity

74-114%