ASPERGILLUS FUMIGATUS

Enzyme immunoassay for the diagnosis of human aspergillosis by *Aspergillus fumigatus*

96 assays on individual wells for in vitro diagnostic use and for professional laboratory use

Instructions for use for article N° **6100**
EC reg. N°: CH-201301-0006

CE

Intended use:

The Bordier Aspergillus fumigatus ELISA kit is intended for the quantitative detection of IgG antibodies against *Aspergillus fumigatus* in human serum. Serology is an aid for diagnosis and cannot be used as the sole method of diagnosis. This test is also intended for follow-up of patients at risk for aspergillosis infections.

Background:

Pulmonary aspergillosis is caused by different pathogenic species of the fungal genus *Aspergillus*, the most frequently implicated being *Aspergillus fumigatus*. This pathogen is found in soil and decaying organic matter. Humans inhale hundreds of spores per day, but only people with risk factors will develop different types of aspergillosis: allergic bronchopulmonary aspergillosis, allergic sinusitis, aspergilloma and chronic pulmonary aspergillosis. The main symptoms are couch and shortness of breath. Since theses symptoms are unspecific, diagnosis is based on a combination of clinical, radiological, biological, and mycological criteria. Serology is an important criterion and several methods are available for screening, follow-up and confirmation.

Principle and presentation:

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with the following mix:

- Solubles somatic and metabolic *Aspergillus fumigatus* antigens
- Recombinants antigens: dipeptidylpeptidase type V (chymotrypsin) and ribonuclease (mitogillin) from *Aspergillus fumigatus*

Specific antibodies in the sample will bind to these antigens and washing will remove unspecific antibodies. The presence of fungal specific antibodies is detected with a Protein A - alkaline phosphatase conjugate. A second washing step will remove unbound conjugate. Revealing bound antibodies is made by the addition of pNPP substrate which turns yellow in the presence of alkaline phosphatase. Color intensity is proportional to the amount of *Aspergillus fumigatus* specific antibodies in the sample. Potassium phosphate is added to stop the reaction. Absorbance at 405 nm is read using an ELISA microplate reader.
The test can be performed with automatic systems, but this must be validated by the user.

Material contained in the kit (96 assays):

WELL	6100-01	Breakable ELISA strips sensitized with ***Aspergillus fumigatus*** antigens	96	wells
DILB	6100-02	Dilution buffer (10 x) concentrate, coloured purple	50	ml
WASH	6100-03	Washing solution (10 x) concentrate	50	ml
ENZB	6100-04	Enzyme buffer	50	ml
STOP	6100-05	Stopping solution (0.5M K_3PO_4)	25	ml
CONTROL -	6100-06	Negative control serum (20 x), green cap	200	µl
CONTROL -/+	6100-07	Weak positive control serum (cut off, 20 x), yellow cap	200	µl
CONTROL +	6100-08	Positive control serum (20 x), red cap	200	µl
CONJ	6100-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap	300	µl
SUBS	6100-10	Phosphatase substrate (para-nitrophenylphosphate)	20	tablets
		Multipipette reservoir, 25 ml	1	piece
		Frame for ELISA 8-well holder	1	piece

Shelf life and storage:

Store the kit at 2° to 8°C (transport at ambient temperature), avoid long term exposure of the components to direct light. The expiry date and the lot number of the kit are printed on the side of the box. After initial opening, all reagents are stable until the expiry date when stored at 2-8°C.

Equipment needed but not provided with the kit:

Pipettes (ml and µl). Flasks. Dilution tubes. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37°C. ELISA reader set at 405 nm. Manual or automatic equipment for rinsing wells. Vortex mixer. Timer.

Preparation of reagents before use:

Bring all reagents to room temperature and mix before use.

ELISA wells: open side of aluminum bag 6100-01 and remove number of wells needed (one for blank, three for controls plus the number of samples). Place sensitized wells in 8-well holder(s). If needed, complete the empty positions in the holder with used wells. Insert holder(s) in the frame in the correct orientation. Reseal open package with desiccant pad.

Dilution buffer: dilute dilution buffer (10 x) concentrate 6100-02, 1/10 in distilled water. This is used for the dilution of controls, samples and conjugate. The diluted buffer is stable for 2 months at 2-8°C.

Washing solution: dilute washing solution (10 x) concentrate 6100-03, 1/10 in distilled water. You may also use your own washing solution. Avoid buffers containing phosphate which could inhibit the enzymatic activity of the alkaline phosphatase. The diluted washing solution is stable for 2 months at 2-8°C.

Control sera: dilute 10 µl control sera 6100-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20). The diluted control sera are stable for 2 months at 2-8°C.

Conjugate: dilute conjugate 6100-09 in dilution buffer solution (final dilution 1/50). Dilute conjugate on the day of the assay. Do not store diluted conjugate.

Substrate solution: dissolve tablet(s) of phosphatase substrate 6100-10 in undiluted enzyme buffer 6100-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s). Dilute substrate on the day of the assay and protect the tube from direct light. Tablets and substrate solutions should be colourless or should have only a slight yellow tinge. If a tablet or a substrate solution turns yellow, it may have been partially hydrolysed and should be discarded. Do not store the substrate solution.

Stopping solution: use reagent 6100-05 undiluted.

Specimen collection and preparation:

Use human serum. Serum should be stored at 2-8°C if analysed within a few days, otherwise store at –20°C or lower. Avoid repeated freezing and thawing.
Vortex samples and dilute 1/201 in dilution buffer solution (for instance 5 µl sample in 1.0 ml).

Warnings and precautions:

Toxic compounds are found in following concentration:

Component	Reference	Sodium azide (N_aN_3)	Merthiolate
Dilution buffer (10 x)	6100-02	0.1 %	0.02 %
Washing solution (10 x)	6100-03	0.05 %	/
Enzyme buffer	6100-04	0.01 %	/
Control sera (20 x)	6100-06 to –08	0.1 %	0.02 %
Conjugate (50 x)	6100-09	0.1 %	/

At the used concentrations, sodium azide and merthiolate do not have any toxicological risk on contact with skin and mucous membranes.

- The stopping solution 6100-05 (0.5 M K_3PO_4) is irritant.

- The negative, weak positive, and positive control sera (6100-06 to -08) are from rabbits.
- Treat all reagents and samples as potentially infectious material.
- Do not interchange reagents of different lots or Bordier ELISA kits.
- Do not use reagents from other manufacturers with reagents of this kit.
- Do not use reagents after their expiry date.
- Close reagent vials tightly immediately after use and do not interchange screw caps to avoid contamination.
- Use separate and clean pipettes tips for each sample.
- Do not re-use microwells.

Disposal consideration:

All materials used for this test are generally considered as hazardous waste. Refer to national and regional laws and regulations for the disposal of hazardous waste.

Procedure:

When running the assay, avoid the formation of bubbles in the wells.

Step 1: Blocking:
Fill completely wells with dilution buffer solution.
Incubate for 5 to 15 minutes at ambient temperature (blocking).
Remove dilution buffer by aspiration or by shaking the wells over the sink.

Step 2: Incubation with samples:
Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank).
Fill the subsequent three wells with respectively 100 µl diluted negative, weak positive (cut off) and positive control serum. For assays of more than 25 samples, we recommend to fill the three last wells with control sera as a duplicate.
Fill remaining wells with the diluted samples (100 µl each).
Cover wells with adhesive tape and incubate for 30 minutes at 37°C.
Remove sera and wash 4 x with ~ 250 µl washing solution.

Step 3: Incubation with conjugate:
Distribute 100 µl diluted conjugate in each well (including no-serum blank).
Cover wells with adhesive tape and incubate for 30 minutes at 37°C.
Remove conjugate and wash 4 x with ~ 250 µl washing solution.

Step 4: Incubation with substrate:
Distribute 100 µl substrate solution per well.
Cover wells with adhesive tape and incubate for 30 minutes at 37°C.
Stop the reaction by the addition of 100 µl stopping solution to each well.

Step 5: Measurement of absorbances:
If needed, wipe the bottom of the wells and eliminate bubbles. Measure absorbances at 405 nm within 1 hour after the addition of stopping solution.

Interpretation:

Subtract the value of the no-serum blank from all measured values. When applicable calculate the mean absorbance values of duplicated serum controls. The test is valid if the following criteria are met:

- absorbance (A) of positive control > 1.200
- A of negative control < 10 % of A of positive control
- A of blank against air < 0.350

Quality controls of current lots are published on our website: www.bordier.ch.

The antibody concentration of the weak positive (cut off) serum 6100-07 has been set to discriminate optimally between sera of clinically documented cases of aspergillosis in immunocompetent patients and healthy human sera.
The cut off index of a sample is defined, after subtraction of the no-serum blank, as:

$$\text{Index} = \frac{\text{Absorbance sample}}{\text{Absorbance cut off serum}}$$

The result is **negative** when the index of the analyzed sample is lower than **0.8**. In this case, the IgG antibody concentration against ***Aspergillus fumigatus*** antigens is clinically non-significant.
A grey area correspond to an index comprised between **0.8** and **1.0**. In this case, the sample is considered as borderline, it is recommended to repeat the test with the same sample or with a new serum of the same patient, taken after 2-4 weeks.
The result is **positive** when the index of the analyzed sample is higher than **1.0**. In this case, the IgG antibody concentration against ***Aspergillus fumigatus*** antigens is considered as clinically significant. This result leads to an aspergillosis or an aspergillosis sensitisation.

Sensitivity and specificity:

A sensitivity of 97% was found with 230 sera from 147 patients suffering from various aspergillosis (104 chronic pulmonary aspergillosis, 17 aspergillomas, and 43 allergic bronchopulmonary aspergillosis). A specificity of 90.3% was found with 206 sera from 205 patients with respiratory symptoms in whom an *Aspergillus*-related disease had been ruled out.

Interferences:

Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

Precision:

Repeatability were assessed by testing 2 human serum samples in 24 wells on 1 assay.
Reproducibility were assessed by testing the 2 human serum samples on 10 differents assays.

	Repeatability		**Reproducibility**	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Average (absorbance)	0.352	1.767	0.410	1.985
Standard deviation (absorbance)	0.027	0.069	0.038	0.096
Variation coefficient (%)	7.6	3.9	9.3	4.8

Limitations:

A sensitivity of 22% was found with 9 sera from 5 patients suffering from invasive aspergillosis. In case of immunosupressed patients, it is recommended to complete the test with the detection of *A. fumigatus* antigens in serum. A specificity of 97% was found with 36 sera from 24 patients suffering from non aspergillus respiratory diseases (candidosis, tuberculosis, pneumocystosis and cryptococcosis).
Diagnosis of an infectious disease should not be estabished on the basis of a single test results. A precise diagnosis should take into consideration endemic situation, clinical history, symptomatology, imaging as well as serological data.
In immunocompromised patients and newborns, serological data are of limited value.

References:

Sarfati, S., Monod, M., Recco, P., Sulahian, A., Pinel, C., Candolfi, E., Fontaine, T., Debeaupuis, J.P., Tabouret, M., Latgé, J.P. (2006) Recombinant antigens as diagnostic markers for aspergillosis. Diag. Microbiol. Inf. Disease **55**, 279-291.
Barrera, C., Richaud-Thiriez, B., Rocchi, S., Rognon, B., Roussel, S., Grenouillet, F., Laboissière, A., Dalphin, J.C., Reboux, G. and Millon, L. (2016) New commercially available IgG kits and time-resolved fluorometric IgE assay for diagnosis of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. Clin Vaccine Immunol **23**, 196 –203.
Dumollard, C., Bailly, S., Perriot, S., Brenier-Pinchart, M.P., Saint-Raymond, C., Camara, B., Gangneux, J.P., Persat, F., Valot, S., Grenouillet, F., Pelloux, H., Pinel, C., Cornet, M. and Grenoble *Aspergillus* Committee. (2016) Prospective evaluation of a new *Aspergillus* IgG enzyme immunoassay kit for diagnosis of chronic and allergic pulmonary aspergillosis. J Clin Microbiol **54**,1236 –1242.

BORDIER AFFINITY PRODUCTS SA
Biokema building, Chatanerie 2, CH-1023 Crissier, Switzerland.
Phone: + 41 21 633 31 67, Fax : + 41 21 633 31 78, www.bordier.ch

51204_05 6100 Eng 01.2018