ASPERGILLUS ANTIGENS

Aspergillus antigens 52942
Aspergillus fumigatus antigens 52962
Aspergillus fumigatus positive control serum 61681
Aspergillus positive control sera 61682

SERODIAGNOSIS OF ASPERGILLOSIS BY IMMUNOPRECIPITATION REACTION
1- **CLINICAL VALUE**

Bio-Rad produces different aspergillus antigens for the serodiagnosis of aspergillosis by immunoprecipitation reaction.

The diagnosis of aspergillosis by the demonstration of specific antibodies in the sera of patients is often easier than diagnosis by direct identification of the fungus.

2- **PRINCIPLE**

The Bio-Rad antigens can be used in different immunoprecipitation reactions. The following two techniques are particularly recommended:

- **IMMUNOELECTROPHORESIS (IEP)**
- **ELECTROSYNERESIS (ES)**

Immunoprecipitation techniques further increase diagnostic precision for aspergillosis through identification of specific antibodies for the two enzymes, chymotrypsin and catalase, present in antigenic extracts. Their enzymatic activity is revealed in precipitation arcs.

3- **PRESENTATION**

- **Aspergillus fumigatus antigens**, code 52962
  2 vials of lyophilized antigens to be reconstituted in 1 ml sterile distilled water (about 10 reactions).
  - Aspergillus fumigatus metabolic antigen
  - Aspergillus fumigatus somatic antigen

- **Aspergillus antigens**, code 52942
  4 vials of lyophilized antigens to be reconstituted in 1 ml sterile distilled water (about 10 reactions).
  - Aspergillus flavus metabolic antigen
  - Aspergillus nidulans metabolic antigen
  - Aspergillus niger metabolic antigen
  - Aspergillus terreus metabolic antigen

- **Aspergillus fumigatus positive control serum**, code 61681
  1 vial of lyophilized anti-Aspergillus fumigatus rabbit serum to be reconstituted in 1 ml sterile distilled water.

- **Aspergillus positive control sera**, code 61682
  4 vial of lyophilized anti-Aspergillus rabbit serum to be reconstituted in 1 ml sterile distilled water.
- Aspergillus flavus positive control serum  
- Aspergillus nidulans positive control serum  
- Aspergillus niger positive control serum  
- Aspergillus terreus positive control serum

4- **COMPOSITION**

Antigens (two types):

- **Metabolic antigen**, obtained by the filtration of cultures after 10 days incubation at 30°C.
- **Somatic** or cellular antigen obtained from young mycelial cultures (3 days, without spores) that are crushed and agitated at 30°C.

5- **STORAGE**

In lyophilized form, reagents stored at +2-8°C, in a dry place, are stable until the expiration date indicated on the kit.

After reconstitution: the reagents can be stored several months at -20°C in divided aliquots. Do not thaw / unthaw the reagents several times.

6- **SPECIMENS**

1. Tests should be performed only on serum samples from dry tubes.
2. Follow these recommendations for the handling, processing, and storing of serum samples:
   - Collect all serum samples observing routine precautions.
   - Allow samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - After centrifugation separate the serum and store it in a tightly stoppered storage tube.
   - The specimens can be stored at +2-8°C if screening is performed within 24 hours. If the assay will not be completed within 24 hours, or for shipment of samples, freeze at -20°C, or colder.
   - Preferably, thaw samples once only. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.
3. Interferences due to high levels of albumin or bilirubin, lipids, hemoglobin have not been tested.
4. Do not heat the samples.
7- PROCEDURE

A) MATERIALS REQUIRED BUT NOT PROVIDED
- Sodium barbital
- Chlorhydric acid
- Distilled water
- Barbital
- Glycine
- Tris buffer 0.05M pH7
- Agarose
- 30% H₂O₂
- N-acetyl-DL phenylalanine naphtyl ester
- Dimethylformamide
- Diazoblué B

B) IMMUNO-ELECTROPHORESIS (photo 1)
Immunoelectrophoresis (Grabar) can be performed on glass slides for microscopic examination.
- Prepare a gel using purified agar or agarose at 1% mixed with barbital buffer solution of pH 8.2 (adding merthiolate 1/10,000).
- Cut in the gel a trough 60 mm long by 2 mm wide and two holes of 2 mm diameter on either side of the trough at distances of 4 mm (see diagram).
- Place 20 µl of undiluted antigen in the two lateral holes.
- For one hour thirty minutes apply a potential of 5 volts/centimeter to the ends of the slide.
- Place the serum in the central trough and allow it to diffuse for 48 hours in a humid atmosphere at ambient temperature (18-30°C).
- On the third day, immerse the slides for 24 hours in a washing solution.
- Read the slide on the 4th day. A Coomassie Blue stain may be necessary in order to read particularly fine lines.

C) ELECTROSYNERESIS (fig. 1)
Electrosyneresis (Bussard) or counter-electrophoresis can be performed using agarose gel or cellulose acetate. It is a simple technique, both very sensitive and rapid, which is useful for screening large numbers of sera.
- Using agarose gel and microscope slides, holes may be cut according to the diagram: antigens placed on the cathode side (-), sera placed on the anode side (+). The holes of 2 mm diameter are separated by 6 mm.
- Fill the antigen holes with pure solutions diluted 1/2 and 1/4. Fill the sera holes with 20 microlitres.
• Apply a 5 volt/centimeter potential for one hour thirty minutes.
• The reaction is usually read immediately after the migration.
• When staining by Coomassie Blue is necessary, it must be preceded by
two successive washings: in physiological serum (during 1 night), then in
distilled water (24 hours), followed by drying.
It is recommended to test in parallel positive controls by following the same
procedure as for the antigens.

D) IDENTIFICATION OF ENZYMATIC ACTIVITY IN CERTAIN
PRECIPITATION ARCS
The arcs most frequently observed after immuno-electrophoresis are
designated C and j (see diagram) and indicate enzymatic activity
(C: chymotripsin and J: catalase). Further evidence for the presence of these
enzymes is provided by specific reactions (BIGUET et al.) (see annex).

E) REAGENT PREPARATION

1) BUFFERS
- VERONAL pH 8,2
  Sodium Barbital 15.85 g
  HCl 1N 23 ml
  H₂O q.s.p. 1000 ml

- BARBITAL- SODIUM BARBITAL TRIS GLYCINE (BBNa TG) pH 8,8
  A. Sodium Barbital 65 g
     Barbital 10,35 g
     H₂O q.s.p. 5000 ml
  B. Glycine 281 g
     Tris 226 g
     H₂O q.s.p. 5000 ml

After mixing equal volumes of the solutions a and b, a buffer is obtained with
a pH of 8.8 and an osmolarity of 0.08.

2) 1% AGAROSE (WEIGHT/VOLUME)
  Agarose 10 g
  H₂O 500 ml

Bring to a boil, then, after cooling (to about 60 °C.)
  Add : Barbital buffer pH 8.2 500 ml

Or
  BBNa TG buffer pH 8,8 500 ml
  (diluted 50% with distilled water).
3) CATALASE
30%H₂O₂, diluted in TRIS 0,05M pH7 buffer (0,3 ml H₂O₂ + 50 ml of buffer) on immuno-electrophoresis agarose.
Positive reaction: bubbles around the specific arc.

4) CHYMOTRYPSIN
- SOLUTION A
  N-acetyl-DL phenylalanine naphtyl ester 5 mg
  Dimethylformamide 2 ml
- SOLUTION B
  Diazobule B 10 mg
  Tris buffer 0,05 M pH 7,4 18 ml

Prior to use mix sol A* & B*, incubate the electrosynersis membrane for 30 minutes at 37°C in this mix.
Eliminate the mix, incubate overnight at 37°C in 18 ml of B reagent.
Wash the the menbranes with water.
Positive reaction: purple coloured arcs correspond to chymotrypsic activity.
8- INTERPRETATION

The presence of precipitation arcs using immunoprecipitation techniques against an Aspergillus antigen indicates infectious aspergillosis. Since it is more rapid, electrosyneresis is preferable to immuno-electrophoresis for screening. However, sera found to be positive using this technique must be confirmed using immuno-electrophoresis. Chymotrypsin and catalase enzymatic activity in certain precipitation arcs provide further evidence of their specificity.

In the case of bronchopulmonary or diffuse aspergillosis, tests are positive in 80 – 90% of cases and specific arcs are generally observed.

* See 6-Procedure (E-Reagents preparation)
In aspergilloma, reactions are highly positive: 5 to 10 arcs and C arc can usually be observed.
In allergic *Aspergillus* bronchitis the reaction is positive in 50% of cases. Precipitating antibody appear late in the infection: in certain cases they are found at the time of a second screening, one month following the first examination.

**9- PERFORMANCES / QUALITY CONTROL OF THE TEST**

**A) DEMONSTRATION OF PRECIPITATION ARCS**
Performances of *Aspergillus* antigens are controlled using aspergillosis positive control sera (anti-*Aspergillus* rabbit serum, obtained by immunizing the animal with mixed standardized *A. fumigatus* antigens and with metabollic standardized *A. flavus, A. niger, A. nidulans* and *A. terreus* antigens).

**Demonstration of precipitation arcs by immuno-electrophoresis (pH = 8.2)**

<table>
<thead>
<tr>
<th>Positive control serum</th>
<th>Anti- <em>A. fumigatus</em></th>
<th>Anti- <em>A. flavus</em></th>
<th>Anti- <em>A. nidulans</em></th>
<th>Anti- <em>A. niger</em></th>
<th>Anti- <em>A. terreus</em></th>
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<tr>
<td><em>A. fumigatus</em> somatic Ag</td>
<td>+ 4 to 8 arcs</td>
<td>-</td>
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<td>+ 3 to 5 arcs</td>
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<td><em>A. flavus</em> metabollic Ag</td>
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<td>+ 3 to 5 arcs</td>
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<tr>
<td><em>A. nidulans</em> metabollic Ag</td>
<td>-</td>
<td>-</td>
<td>+ 3 to 5 arcs</td>
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<td>-</td>
</tr>
<tr>
<td><em>A. niger</em> metabollic Ag</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ 3 to 5 arcs</td>
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<tr>
<td><em>A. terreus</em> metabollic Ag</td>
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<td>-</td>
<td>-</td>
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<td>+ 3 to 5 arcs</td>
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10- QUALITY CONTROL OF THE MANUFACTURER
All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

11- LIMITS OF USE
Aspergillosis diagnosis can only be made after consideration of clinical, radiological and biological (microbiological, histological, serological) aspects, each element taken separately having to be interpreted with caution.
12- REFERENCES


5. SEGRETAIN G.; DROUHET ; MARIAT F.; Diagnostic de laboratoire en mycologie médicale (1979, 4e édition), MALOINE Edt. PARIS 150 pages.
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The other languages which are required in conformity to the European Directive can be obtained from your local Bio-Rad agent.

Les autres langues requises par la Directive Européenne sont disponibles auprès de votre représentant Bio-Rad local.

Los otros idiomas que se requieren para la conformidad de la Directiva Europea puede ser obtenida en su oficina local Bio-Rad.

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