Kasmera 31(1): 29 - 31, 2003
ISSN 00755222 / Depósito legal 196202ZU39

Modification of the ID-Fungal (IMMY) in Immune-Diagnosis of Deep Mycosis

Modificaciones en el Sistema ID-Fungal (IMMY) en el inmunodiagnóstico de micosis profundas

Magaldi, Sylvia[1]; Reyes, Heidi[2]; Hartung de Capriles, Claudia[1]; Mata-Essayag, Sofía[1]; Pérez, Celina[1] & Colella, María Teresa[1]

[1]Sección de Micología Médica, Instituto de Medicina Tropical, UCV. E-mail: smagaldi@cantv.net. [2]Laboratorio de Micología, Hospital "Domingo Luciani", Caracas.

Resumen

Se describe en esta nota técnica las modificaciones realizadas al sistema ID-Fungal anticuerpos de la línea IMMY para serología de hongos. De las cuales la primera se relaciona con el incremento de la sensibilidad de la técnica a través de la concentración del suero, duplicando la cantidad del mismo cuando este es añadido a los pozos de difusión. Este procedimiento es útil principalmente en pacientes inmunocomprometidos en los cuales la cantidad de anticuerpos en sangre es baja. Con la segunda modificación se pretende facilitar la lectura de las bandas de precipitación mediante una coloración con azul brillante de Coomassie, lo cual a su vez permite la preservación y almacenaje de las placas en el tiempo.

Palabras clave: IMMY, inmunodifusión, anticuerpos, micosis profundas.

Abstract

This technical report describes the modifications made in ID-Fungal anti-bodies in the IMMY line in fungus serology. The first part is related to the increase in the sensitivity of the technique due to the concentrations of serum, which doubles the quantity of the same when added to diffusion wells. This procedure is useful basically in immune-compromised patients in which the quantity of blood antibodies is low. The second modification helps to facilitate the reading of precipitation bands through bright blue coloring with Coomassie, which at the same time aids the preservations and storage of the plates over time.

Key words: IMMY, immune-diffusion, antibodies, deep mycosis.

Recibido: 04-11-02 / Aceptado: 26-11-02

Brief Report

The ID-Fungal Antibody System, catalog Nº ID1001, Immuno-Mycologics, Inc.[1], is composed of qualitative screening tests for the detection of precipitating antibodies in patient sera as an aid in the diagnosis of histoplasmosis, blastomycosis, coccidioidomycosis, and aspergillosis. The tests are based upon the principles of immunodiffusion or double diffusion as originally described by Oudin [2] and Ouchterlony [3]. Antibodies and soluble antigens are placed into separate wells cut in the surface of a suitable diffusion medium (eg. Agar, agarose, polyacrylamide, gelatin, Cleargel ™, etc.) and allowed to diffuse outward in the medium. Visible lines of precipitate are formed in the diffusion medium where the antigens and antibodies have combined in the proper relative concentrations.

The aim of this short communication is to suggest improvements for the use of the ID-fungal antibody system (Catalog No. ID1001) on a daily basis.

Performing the test as shown in Catalog No. ID1001 ID-fungal antibody system:

1. Label the ID plates to be used with an identifying number and the date. Place the plates on a dark background for well filling.
2. Using capillaries and the rubber bulb, fill the control serum wells of the ID plates with the appropriate control sera as follows:
   a) *Histoplasma* Control Serum- Series I, wells 1&4.
   b) *Blastomyces* Control Serum-Series II, wells 1 & 4.
   c) *Coccidioides* control Serum-Series III, wells 1 & 4.
   d) *Aspergillus* Control Serum-Series IV, wells 1 & 4.
3. Record the plate number and date on the reading form.
4. Record the name, date, and/or lab number of the first patient on line 2 of the left hand column of the reading form.
5. Using a capillary tube, fill well 2 of Series I,II,III and IV of the plate with the first patient specimen.
6. Repeat steps 4 and 5 with each additional patient specimen using well 3,5 and 6 of Series I,II,III and IV.
7. After adding the control sera and patient sera the closed plate may be incubated at room temperature for 30 minutes. This will cause the bands to be slightly more intense than if the antigens are added immediately.
8. Using a capillary, fill the center well (#7) of Series I with *Histoplasma* Antigen. Repeat the process of filling the center wells with *Blastomyces* Antigen (Series II). *Coccidioides* Antigen (Series III), and *Aspergillus* Antigen (Series IV).
9. Place the ID plates in a moist chamber, well leveled, and incubate at room temperature for 24 hours (some patient reactions with *Blastomyces* Antigen may not appear until 48 hours of incubation).

To improve the performance of these test, specially in those patient sera, where antibodies may be present in less quantity (immunocompromised patients, patients in the early phase of primary infection etc.),concentration of the serum in step Nº 7 may be advisable, as well as extending the incubation time to 48h according to step Nº 9 in the protocol. The sera may be concentrated, by doubling the quantity, this can be done by adding the sera a second time after the first addition had been adsorbed, after approximately 2-3min.

The catalog does not contemplate coloring of the gel for reading the test, and we

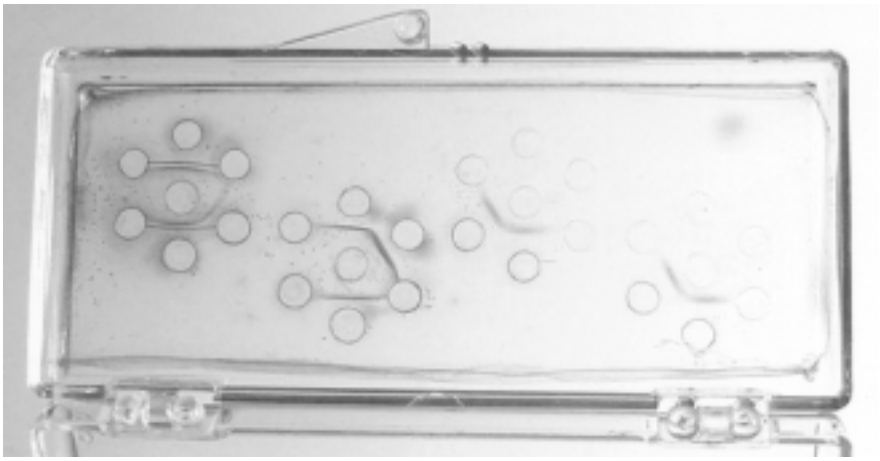

Figure 1. Coomassie blue stained ID-Fungal Antibody System plate.

herein present an additional procedure, that may allow coloring the gel for easier reading, and also avoid false positives by eliminating unspecific precipitin bands on the ID plates, as well as preserve them for storage (Figure 1).

Coloring procedure

10. After incubation time of 24-48 h has been completed, the plates will be continually flooded with 5% sodium citrate (pH 7.0), allowing the plates to stand at room temperature for 90 minutes.
11. After completing 90 minutes the plates will be washed with water and flooded with 0.85% sodium chloride for 24 h.
12. Drying procedure: to avoid cracking of the gel during the drying process, it is advisable to cover with a humidified filter paper or to put an absorbent wetted paper in the bottom and let it dry gradually at room temperature (27-29°C in our lab). It may be dried faster at 56°C in an incubator, wetting the covering filter paper from time to time to avoid cracking of the gel reducing the drying time to 3-6 h if necessary.
13. Staining will be done with Coommassie Brilliant blue R250 (2.5 g in 500 mL solvent solution (1125 mL 95% ethanol; 250 mL glacial acetic acid; 1125 mL distilled water) for 10 minutes.
14. Washing of the gel plates will be performed by successive submerging of the plates in solvent solution until the bands are clearly observable.

We are currently performing these procedures on a routine basis and so far have had excellent results in our lab. We hope this suggestions may be useful for other mycological laboratories.

References

(1) IMMY ID-Fungal Antibody System Catalog No. ID 1001 Immuno-Mycologics, Inc. f:\apps\pkgins\id10011.p65.

(2) Oudin, J. 1984. Lánalyse, immunochemique qualitative, Mehode par diffusion des antigens au sein de Límmuno-serum precipitant gelose. Premiere Parte. Inst. Pasteure 75: 30-52

(3) Ouchterlony, O. 1968. Handbook of immunodiffusion and immunoelectrophoresis. Ann Arbor Science Publishers. Inc.