INTENDED USE

The Immunodiffusion (ID) reagents are designed to detect patient antibodies directed against Aspergillus, Blastomyces, Candida, Coccidioides, Histoplasma, or Paracoccidioides in patient serum to aid in the diagnosis of each respective disease.

EXPLANATION

Aspergillus spp., Blastomyces dermatitidis, Candida spp., Coccidioides immitis, Coccidioides posadasii, Histoplasma capsulatum, and Paracoccidioides brasiliensis are causative agents of deep-seated mycoses. These fungi present a diagnostic challenge to the microbiology laboratory and physician. Radiographically, lesions produced by the systemic fungi (all of the above except Candida) may be difficult to distinguish from other infectious diseases (e.g. M. tuberculosis) or neoplastic diseases (23). Symptoms are often unremarkable and may mimic various pneumonias, sarcoidosis, cancer and other maladies. Culturally and histologically the organisms may be difficult to demonstrate, even after repeated attempts. With the exception of Aspergillus and Candida, growth is very slow, requiring 2-6 weeks (26). Frequently, serology offers the only evidence available to guide treatment, suggest prognosis or lead to the selection of more definitive diagnostic techniques such as intensive culture or biopsy (23). In addition, semi-quantitative serology, such as complement fixation (CF) testing or semi-quantitative ID, can provide important information on the effects of therapy (23,24). The ID test is a qualitative or semi-quantitative test employed for the detection of precipitating antibodies from patients suspected of having mycoses. Additionally, the ID test is a rapid and reliable tool that provides presumptive evidence of infection. Anti-complementary sera in the CF test may be tested using this technique. The ID test also provides specificity data on reactions obtained by the CF test. No expensive equipment is required and the technique is simple enough to be performed by any laboratory, thereby providing an excellent screening tool.

<table>
<thead>
<tr>
<th>Disease</th>
<th>ID</th>
<th>CF</th>
<th>LA</th>
<th>EIA</th>
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<tbody>
<tr>
<td>Aspergillosis</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Blastomycosis</td>
<td>X</td>
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<tr>
<td>Candidiasis</td>
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<td>Coccidioidomycosis</td>
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<td>Histoplasmosis</td>
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<tr>
<td>Paracoccidioidomycosis</td>
<td>X</td>
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</tbody>
</table>

ASPERGILLOSIS:

There are three forms of aspergillosis: invasive aspergillosis (IA), allergic bronchopulmonary aspergillosis (ABPA), and aspergilloma (19). The ID test is very helpful in the diagnosis of ABPA and aspergilloma, the two forms of aspergillosis observed in immunocompetent individuals where infection can be correlated with a rise in specific antibodies (19). Precipitins can be found in >90% of patients with aspergilloma and in 70% of patients with ABPA (26). In contrast to immunocompetent hosts, growth of Aspergillus in the tissues of an immunosuppressed host does not correlate with an increase in anti-Aspergillus antibody titers. The parallel use of ID and CF tests and clinical data is an effective means for specific diagnosis of Aspergillosis (Table 1) (33).

BLASTOMYCOSIS:

Serologic testing for Blastomycosis should be requested when a patient shows signs of a respiratory infection that progresses gradually and/or when lesions are present on the skin, a frequent sign of dissemination (26). Blastomycosis has no pathognomonic symptoms or specific radiologic features. The ID test for Blastomycosis where antibodies are detected against the “A” antigen is specific (positive in approximately 80% of culturally proven cases) and positive reactions can be the basis for immediate treatment of the patient (26). The amount of antibody (i.e. titer) correlates with disease activity (7). Negative tests, however, do not exclude diagnosis of Blastomycosis (26). If precipitins are demonstrated in the Blastomycosis ID test, the testing of serum specimens by CF is unnecessary (Table 1) (34).
CANDIDIASIS:
*Candida* species, although usually considered saprophytic, are now known to cause disease. Current treatment regimens such as broad-spectrum antibiotics, corticosteroids and cytotoxic drugs have led to an increased prevalence of systemic Candidiasis. Unfortunately, unique clinical features are lacking in visceral Candidiasis and the diagnosis is frequently not made antemortem. Early and accurate diagnosis is the key to successful therapy. Stallybrass (30) has reported the significance of precipitating antibodies against *C. albicans* in sera from patients with systemic or visceral candidiasis using the ID test. Sera from healthy individuals and patients with mucocutaneous candidiasis did not give positive ID tests. The ID test for antibodies is helpful in the diagnosis of systemic candidiasis in immunocompetent hosts (26). However, immunosuppressed patients may mount a limited humoral immune response or fail to produce antibodies, so a negative ID test does not rule out disease (26). Because these fungi occur as part of the normal flora of the body, their isolation may not be sufficient evidence for laboratory diagnosis. Serologic tests provide additional information where a rise in titer or change in the number of precipitins is more indicative of infection than a single positive test.

COCCIDIOIDOMYCOSIS:
*C. immitis* (California isolates) and *C. posadasii* (non-California isolates) are found predominately in the southwestern United States (11), and Central and South America, but modern transportation has increased the likelihood of infection for individuals visiting the region. Serologic diagnosis of Coccidioidomycosis is generally based on the detection by ID and complement fixation (CF) of antibodies to two *Coccidioides* antigens, IDTP and IDCF (24). IDTP antibodies have been associated with primary acute disease and are thought to belong mostly to the IgM class (24). IDCF antibodies persist during the chronic, disseminated phase of the disease and have been described as primarily IgG antibodies (32). However, IDTP and IDCF antibodies may not be detected in immunocompromised or immunosuppressed patients (4,5,8). The CF test, when run concurrently with the ID test, is the best combination for making a presumptive diagnosis of Coccidioidomycosis (Table 1) (34). The semi-quantitative IDCF test titer is not identical to the CF test titer, but the trends are comparable and have prognostic value (24).

HISTOPLASMOSIS:
Histoplasmosis results from infection with *H. capsulatum*, which has a worldwide distribution but is a particular problem in central and southeastern areas of the United States and in certain regions of Central and South America (31). Two precipitins can be detected in Histoplasmosis patient serum directed against the "M" and "H" antigens. Antibodies against "M" antigen are the first to appear in acute pulmonary Histoplasmosis (10) and form the basis of a specific immunodiagnosis, while antibodies to "H" antigen occur later and less frequently (27), and their presence is often linked to extrapulmonary dissemination. Approximately 63% of culturally confirmed Histoplasmosis cases have only an "M" band, whereas, 90% have either an "M" band only or "H" and "M" bands in the ID test (6). The Histoplasmosis ID and CF tests will react with about 85 - 94% of sera from patients with Histoplasmosis (6,16). Antibody titers are of diagnostic and prognostic value (26). The parallel use of ID and CF tests and clinical data is an effective means for specific diagnosis of Histoplasmosis (Table 1) (34).

PARACOCCIDIOIDOMYCOSIS:
*P. brasiliensis* is the causative agent of Paracoccidioidomycosis, which is endemic in Central and South America (9). Paracoccidioidomycosis in AIDS patients is relatively rare (12). Therefore, detection of antibody is a useful diagnostic and prognostic tool (9). The main diagnostic precipitin is gp43 (also known as E2 or A), which is found in 95-98% of patients with Paracoccidioidomycosis (9).

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**Figure 1.**

- **Identity**
  - Ag (Antigen)
  - PC (Patient)
  - PT (Precipitin)

- **Non Identity**
  - Ag
  - PC
  - PT

- **Partial Identity**
  - Ag
  - PC
  - PT

*PT= Patient
*Ag= Antigen*
BIOLOGICAL PRINCIPLES

Immunodiffusion is a qualitative or semi-quantitative test based on the principles of double diffusion described by Oudin (22) and Ouchterlony (20,21). An antibody and its homologous soluble antigen are placed in separate wells cut in a suitable diffusion medium (agarose or Cleargel™) and allowed to diffuse outward into the medium. Between the two wells, a concentration gradient of each of the reaction components is established ranging from antigen excess closest to the antigen well, to antibody excess closest to the antibody well. A visible line of precipitate forms at the point of equivalence.

Patient antibodies are tested for “identity” by placing patient serum adjacent to the wells of a known reference system. Additionally, the patient specimen is placed adjacent to a positive control to obtain maximum sensitivity (i.e. low positive specimens will show a turning of the reference band when placed adjacent to a positive control). If an antigen-antibody complex is identical, a precipitin line forms an unbroken line of identity with the known reference system. The well patterns of the immunodiffusion plate are arranged to provide each patient test with a known reference system so that identity reactions are readily apparent (Figure 1). Partial identity and nonidentity reactions are also possible. (Figure 1).

A partial identity reaction occurs when certain components of the antibodies are identical and others are not. Partial identity reactions indicate the simultaneous occurrence of both an identity reaction and a non-reaction. The “spur” represents the components that are unrelated. A nonidentity reaction will occur when the antigen-antibody complexes are different. The resulting “X” or crossed reaction indicates that two unrelated complexes are present.

MATERIALS PROVIDED

All reagents are intended for in vitro diagnostic use only!

Materials Supplied (Dependent upon which kit or reagents are purchased)

A. ID Antigens:

1. **Aspergillus ID Antigen** (0.2 ml, REF A30000; 1.0 ml, REF A30110) Pooled mycelial-phase culture filtrates of *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*.
2. **A. fumigatus ID Antigen** (1.0 ml, REF A70110) Mycelial-phase culture filtrate of *A. fumigatus*.
3. **A. flavus ID Antigen** (1.0 ml, REF A90110) Mycelial-phase culture filtrate of *A. flavus*.
4. **A. niger ID Antigen** (1.0 ml, REF AD0110) Mycelial-phase culture filtrate of *A. niger*.
5. **A. terreus ID Antigen** (1.0 ml, REF AF0110) Mycelial-phase culture filtrate of *A. terreus*.
6. **Blastomyces ID Antigen** (0.2 ml, REF B30000; 1.0 ml, REF B30110) A purified extract of the yeast-phase growth of *B. dermatitidis* containing the “A” antigen.

7. **Candida ID Antigen** (0.25 ml, REF C50000; 1.0 ml, REF C50110) Culture filtrate and cell lysate preparation of yeast-phase growth of *C. albicans* serotype A.
8. **Coccidioides IDCF Antigen** (0.2 ml, REF C30000; 1.0 ml, REF C30110) Mycelial-phase culture filtrate of *C. immitis* containing the “IDCF” antigen. This preparation may also contain the “IDTP” antigen.
9. **Coccidioides IDTP Antigen** (1.0 ml, REF C70110) Mycelial-phase culture filtrate of *C. immitis* containing the “IDTP” antigen.
10. **Histoplasma ID Antigen** (0.2 ml, REF H50000; 1.0 ml, REF H50110) Mycelial-phase culture filtrate of *H. capsulatum* containing “H” and “M” antigens.
11. **Paracoccidioides ID Antigen** (1.0 ml, REF PI0110) Mycelial-phase culture filtrate of *P. brasiliensis* containing “gp43” and other antigens.

B. ID Positive Controls (Goat anti-sera)

1. **Aspergillus ID Positive Control** (0.4 ml, REF A40000; 1.0 ml, REF A40110) Contains antibodies directed against *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. At least two bands are apparent against Aspergillus ID antigen (REF A30000 and A30110).
2. **A. fumigatus ID Positive Control** (1.0 ml, REF A80110) Contains antibodies directed against *A. fumigatus*. At least two bands are apparent against the *A. fumigatus* ID antigen (REF A70110).
3. **A. flavus ID Positive Control** (1.0 ml, REF AA0110) Contains antibodies directed against *A. flavus*. At least one band is apparent against the *A. flavus* ID antigen (REF A90110).
4. **A. niger ID Positive Control** (1.0 ml, REF AE0110) Contains antibodies directed against *A. niger*. At least one band is apparent against the *A. niger* ID antigen (REF AD0110).
5. **A. terreus ID Positive Control** (1.0 ml, REF AG0110) Contains antibodies directed against *A. terreus*. At least one band is apparent against the *A. terreus* ID antigen (REF AF0110).
6. **Blastomyces ID Positive Control** (0.4 ml, REF B40000; 1.0 ml, REF B40110) Contains antibodies directed against *B. dermatitidis* “A” antigen. One band is apparent against Blastomyces ID antigen (REF B30000 and B30110).
7. **Candida ID Positive Control** (0.5 ml, REF C60000; 1.0 ml, REF C60110) Contains antibodies directed against *C. albicans* serotype A. At least two bands are apparent against Candida ID antigen (REF C50000 and C50110).
8. **Coccidioides IDCF Positive Control** (0.4 ml, REF C40000; 1.0 ml, REF C40110) Contains antibodies directed against the Coccidioides IDCF ID antigen (REF C30000 and C30110). The band closest to the antigen well is IDCF. If apparent, the IDTP band is closest to the positive control well.
9. **Coccidioides IDTP Positive Control** (1.0 ml, REF CD0110) Contains antibodies directed against the Coccidioides IDTP ID antigen (REF C70110). One band is apparent against Coccidioides IDTP antigen.
10. **Histoplasma ID Positive Control** (0.4 ml, REF H60000; 1.0 ml, REF H60110) Contains antibodies directed against *H. capsulatum* "H" and "M" antigens. Two bands are apparent against Histoplasma ID antigen (REF H50000 and H50110). The "H" band is closest to the positive control well and the "M" band is closest to the antigen well.

11. **Paracoccidioides ID Positive Control** (1.0 ml, REF PJ0110) Contains antibodies directed against *P. brasiliensis* "gp43" and other antigens. At least one band is apparent against Paracoccidioides ID antigen (REF PI0110).

**Note:** The 0.2 ml size of antigen and the 0.4 ml and 0.5 ml sizes of positive controls are only available in kits and may not be purchased independently.

C. **ID Plates:**
   1. Cleargel™ 1-Series, 10/pk (REF CA1019)
   2. Cleargel™ 4-Series, 6/pk (REF ID1019)
   3. Cleargel™ 4-Series, 6/pk – Large well (REF ID1039)
   4. Agarose 4-Series, 6/pk (REF ID1029)

(ID plates dry easily. Keep bags sealed tightly. DO NOT FREEZE. Store at 2-8°C.

**Note:** Cleargel plates are not to be used with rabbit antiserum

D. **Plate Forms:** Plate forms for recording patient data and test results.

**MATERIALS NOT PROVIDED**

1. Distilled or DI water.
2. Moist Chamber: Any convenient container may be used that has a tight fitting cover (e.g. petri plate, plastic box, large screw-capped jar) and contains moist filter paper or paper toweling, provided that ID plates remain stationary, level, and hydrated during incubation.
3. Reading Light: A high-intensity light (VWR Cat# 41447-193 or comparable) is used to read the ID reactions.
4. Pipettor and tips: Pipettor and tips capable of delivering 20-35 μl are required for filling the ID plate wells.
5. Phosphate Buffered Saline (PBS) or normal saline can be used for dilution of patient specimens for the semi-quantitative ID test.

**STABILITY AND STORAGE**

Fungal antigens should be stored at 2-8°C and are stable until the expiration date. The positive control serum is stable until the expiration date when stored at 2-8°C PRIOR to rehydration. The rehydrated positive control will remain stable for up to one month if stored at 2-8°C. For storage periods longer than one month, the rehydrated positive control serum should be aliquotted and frozen where it will remain stable until the expiration date. Repeated freezing and thawing should be avoided. When the positive control sera are in use, the period at room temperature should be kept as short as possible. ID plates are stable until their expiration date when properly stored at 2-8°C in their zip-loc bags to prevent drying (indicated by increasing cloudiness). ID plates must NEVER be frozen.

![Figure 2](image-url)
PRECAUTIONS

All fungal ID reagents are for in vitro diagnostic use only. Specific standardization is necessary to produce our high quality reagents and materials. IMMY cannot guarantee the performance of its products when used with materials purchased from other manufacturers. The user assumes full responsibility for any modification to the procedures published herein. When handling patient specimens, adequate measures should be taken to prevent the exposure to etiologic agents potentially present in the specimen. The positive control sera are preserved with 0.095% w/w sodium azide. It is therefore recommended that excess positive control simply be discarded in an appropriate waste receptacle.

REAGENT PREPARATION

Rehydration of Positive Controls: To 0.4 ml size of positive control add 0.4 ml of distilled or DI water; to 0.5 ml size add 0.5 ml; to 1 ml sizes add 1 ml. Incubate at room temperature until completely dissolved, followed by gentle mixing.

SPECIMEN PREPARATION

For optimal results, sterile serum is used, which should not be severely lipemic or contaminated. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. Specimens may be stored for longer periods at < -20°C, provided they are not repeatedly thawed and refrozen. Specimens in transit between laboratories should be maintained at 2-8°C for optimal results. Specimens may be preserved with 0.01% thimerosal or 0.095% sodium azide if necessary.

PROCEDURE

ID Fungal Antibody System Procedure (REF ID1001)

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. Fill the positive control wells (See Figure 2) of the ID plates with the appropriate positive control sera as follows:
   A. Histoplasma ID Positive Control– Series I, wells 1 & 4.(H6)
   B. Blastomyces ID Positive Control– Series II, wells 1 & 4.(B4)
   C. Coccidioides ID Positive Control– Series III, wells 1 & 4.(C4)
   D. Aspergillus ID Positive Control– Series IV, wells 1 & 4.(A4)

NOTE: To properly fill the wells, fill the well until the edge of the well disappears. Pay special attention not to over- or under- fill the wells.

3. Record the plate number, lot #, and date for the plate on the plate form
4. Record the name, date, and/or lab number of the first patient on line 2 of the left hand column of the plate form
5. Fill well 2 of Series I, II, III, and IV of the plate with the first patient specimen.
6. Repeat step 4 and 5 with each additional patient specimen using wells 3, 5 and 6 of Series I, II, III, and IV.
7. After adding the positive control and patient sera, the closed plate may be pre-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. Fill the center well (#7) of Series I with Histoplasma (H5) ID Antigen. Repeat the process of filling the center wells with Blastomyces (B3) ID Antigen (Series II), Coccidioides (C3) ID Antigen (Series III), and Aspergillus (A3) ID Antigen (Series IV).
9. Place the closed ID plates level, in a moist chamber and incubate at room temperature for 24 hours.
10. After 24 hours, read and record the ID bands on the analysis form. See Reading the Test. An interim report should be issued at this point if no identity or partial-identity reactions are observed. Positive results should be reported immediately. If control bands fail to appear in 24 hours, then repeat the test.
11. An additional 24 hours is recommended to confirm a negative result. A final report is made at the conclusion of this period. (Some patient reactions with Histoplasma and Blastomyces ID Antigens may not appear until 48 hours of incubation).

Aspergillus species, ID-Candida Antibody System (REF CA1001), Paracoccidioides Immunodiffusion Procedure, Individual reagents, and Individual Plates

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 the appropriate ID Positive Control, fill wells 1 & 4 of the ID plates with the ID Positive Control.

NOTE: To properly fill the wells, fill the well until the edge of the well disappears. Pay special attention not to over- or under fill the wells.

3. Record the plate number, lot #, and date for the plate on the plate form
4. Record the name, date, and/or lab number of the first patient on line 2 of the left hand column of the plate form
5. Fill well 2 of the ID plate with the first patient specimen.
6. Repeat steps 4 and 5 with each additional patient specimen using wells 3, 5 and 6.
7. After adding the positive control and patient sera, the closed plate may be pre-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. Fill the center well (#7) with the homologous ID Antigen. Repeat the process of filling the center wells using wells 3, 5 and 6 of Series I, II, III, and IV.
9. Place the closed ID plates level, in a moist chamber and incubate at room temperature for 24 hours.
10. After 24 hours read and record the ID bands on the analysis form. See Reading the Test. An interim report should be issued at this point if no identity or partial-identity reactions are observed. Positive results should be reported immediately. If control bands fail to appear in 24 hours, then repeat the test.
11. An additional 24 hours is recommended to confirm a negative result. A final report is made at the conclusion of this period.

SPECIMEN PREPARATION

For optimal results, sterile serum is used, which should not be severely lipemic or contaminated. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. Specimens may be stored for longer periods at < -20°C, provided they are not repeatedly thawed and refrozen. Specimens in transit between laboratories should be maintained at 2-8°C for optimal results. Specimens may be preserved with 0.01% thimerosal or 0.095% sodium azide if necessary.

PROCEDURE

ID Fungal Antibody System Procedure (REF ID1001)

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. Fill the positive control wells (See Figure 2) of the ID plates with the appropriate positive control sera as follows:
   A. Histoplasma ID Positive Control– Series I, wells 1 & 4.(H6)
   B. Blastomyces ID Positive Control– Series II, wells 1 & 4.(B4)
   C. Coccidioides ID Positive Control– Series III, wells 1 & 4.(C4)
   D. Aspergillus ID Positive Control– Series IV, wells 1 & 4.(A4)

NOTE: To properly fill the wells, fill the well until the edge of the well disappears. Pay special attention not to over- or under- fill the wells.

3. Record the plate number, lot #, and date for the plate on the plate form
4. Record the name, date, and/or lab number of the first patient on line 2 of the left hand column of the plate form
5. Fill well 2 of Series I, II, III, and IV of the plate with the first patient specimen.
6. Repeat step 4 and 5 with each additional patient specimen using wells 3, 5 and 6 of Series I, II, III, and IV.
7. After adding the positive control and patient sera, the closed plate may be pre-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. Fill the center well (#7) of Series I with Histoplasma (H5) ID Antigen. Repeat the process of filling the center wells with Blastomyces (B3) ID Antigen (Series II), Coccidioides (C3) ID Antigen (Series III), and Aspergillus (A3) ID Antigen (Series IV).
9. Place the closed ID plates level, in a moist chamber and incubate at room temperature for 24 hours.
10. After 24 hours, read and record the ID bands on the analysis form. See Reading the Test. An interim report should be issued at this point if no identity or partial-identity reactions are observed. Positive results should be reported immediately. If control bands fail to appear in 24 hours, then repeat the test.
11. An additional 24 hours is recommended to confirm a negative result. A final report is made at the conclusion of this period.

Aspergillus species, ID-Candida Antibody System (REF CA1001), Paracoccidioides Immunodiffusion Procedure, Individual reagents, and Individual Plates

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 the appropriate ID Positive Control, fill wells 1 & 4 of the ID plates with the ID Positive Control.

NOTE: To properly fill the wells, fill the well until the edge of the well disappears. Pay special attention not to over- or under fill the wells.

3. Record the plate number, lot #, and date for the plate on the plate form
4. Record the name, date, and/or lab number of the first patient on line 2 of the left hand column of the plate form
5. Fill well 2 of the ID plate with the first patient specimen.
6. Repeat steps 4 and 5 with each additional patient specimen using wells 3, 5 and 6.
7. After adding the positive control and patient sera, the closed plate may be pre-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. Fill the center well (#7) with the homologous ID antigen. Repeat the process of filling the center wells using wells 3, 5 and 6 of Series I, II, III, and IV.
9. Place the closed ID plates level, in a moist chamber and incubate at room temperature for 24 hours.
10. After 24 hours read and record the ID bands on the analysis form. See Reading the Test. An interim report should be issued at this point if no identity or partial-identity reactions are observed. Positive results should be reported immediately. If control bands fail to appear in 24 hours, then repeat the test.
11. An additional 24 hours is recommended to confirm a negative result. A final report is made at the conclusion of this period.
Semi-Quantitative ID Procedure:
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 the appropriate ID Positive Control, fill wells 1 & 4 of the ID plates.

**NOTE:** To properly fill the wells, fill the well until the edge of the well disappears. Pay special attention not to over or under fill the wells.

3. Record the plate number and date for the plate on the plate form.
4. Record the name, date, and/or lab number of the first patient on line 2 of the left hand column of the plate form.
5. Fill well 2 of the ID plate with the first patient specimen undiluted.
6. Repeat steps 4 and 5 with each additional dilution of patient specimen using wells 3, 5 and 6. Two-fold serial dilutions of the patient specimen can be performed with PBS or normal saline.
7. After adding the positive control 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. Fill the center well (#7) with the homologous ID antigen.
9. Place the closed ID plates level, in a moist chamber and incubate at room temperature for 24 hours.
10. After 24 hours read and record the ID bands on the analysis form. See **Reading the Test**. An interim report should be issued at this point if no identity or partial-identity reactions are observed. Positive results should be reported immediately. If control bands fail to appear in 24 hours, then repeat the test.
11. An additional 24 hours is recommended to confirm a negative result. A final report is made at the conclusion of this period.

**Reading the Test**
The precipitin bands on the ID plate may be easily read in a beam of high-intensity light with the plate held over a dark background and the light projecting through the plate from below at approximately 45° to the surface of the plate. The eye of the person reading should be above the plate, outside the beam of light in such a position that light reflecting off of the bands makes them appear bright. Rotating the ID plates may help identify weak positive ID reactions. Record on the reading form all bands observed on the plates.
The control bands, as previously described in section B “ID Positive Controls”, must be present in order for the patient tests to be valid. If any bands are missing the test should be repeated. Particular attention should be paid to the orientation of bands produced by the patient serum in relation to the control bands. The ends of the control bands should be carefully observed. A smooth junction of the bands is indicative of an identity reaction and a junction with a spur is indicative of a partial-identity reaction (Figure 1). If the control is seen to bend toward a position in front of the patient well, it is indicative of a patient antibody at a low titer. It is recommended that weak positive specimens be set-up with the positive control in well 1, patient specimen in well 2, Negative Control (Cat # N80110) or a negative specimen in well 6, and antigen in well 7. This set-up will aid in confirming a weak positive result.
Partial identity bands contain both an identity band and a non-reaction band and are therefore considered positive because of the identity band.
Non-identity bands against *Aspergillus* ID Antigen should be tested to determine whether or not they are due to C-reactive protein. This false-positive reaction can be eliminated by soaking the plate in 5% sodium citrate for approximately 45 minutes at room temperature followed by a DI water rinse and reapplication of 5% sodium citrate for an additional 45 minutes before reading the reaction.

**INTERPRETATION OF RESULTS**

Bands of identity or partial identity with a Positive Control are considered positive and indicate patient antibody against the antigen in question. Absence of bands or non-identity reactions are regarded as a negative test (1-3); however, non-identity reactions with *Aspergillus* or *Candida* should make one suspect Aspergillosis or Candidiasis. Although a specific diagnosis cannot be made in the absence of identity or partial identity reactions, the number of bands should be reported.
LIMITATIONS OF THE PROCEDURE

The greatest limitation of the test procedure is with specimens from patients with early, primary infections (first 3-6 weeks). Additionally, immunocompromised or immunosuppressed patients may not produce detectable amounts of antibody.

EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS

ASPERGILLOSIS:

Precipitins can be found in >90% of patients with aspergillomas and in 70% of patients with ABPA (26). The greatest number of Aspergillosis cases may be detected by the use of A. fumigatus, A. flavus, A. niger, and A. terreus antigens in separate ID tests performed at the same time (26). Precipitins are less frequent in patients with invasive Aspergillosis. Some Aspergillus spp. antigenic extracts contain C substance, and this can react with C-reactive protein in the serum of some patients with inflammatory disease. The resulting complex forms a precipitate, which may be erroneously interpreted as being to Aspergillus antibodies (26). In fact, the presence of anti-Aspergillus antibody in immunocompromised individuals is more likely to represent antibody formed before the onset of immunosuppressive therapy rather than as a result of invasive infection. An increase in antibody titer at the end of immunosuppression is indicative of recovery from IA, whereas absence of an antibody titer or declining antibody levels suggest a poor prognosis. Thus, antibody detection can be used prognostically but not diagnostically for IA (19). Two or more distinct precipitin lines should be formed when A. fumigatus reference antiserum is allowed to react with A. fumigatus antigen. One or more distinct precipitin lines should be formed when A. flavus, A. niger, or A. terreus reference antiserum is allowed to react with the homologous antigen. Because the Aspergillus antigens of diagnostic significance have not been defined, any precipitin band (whether identity, partial identity, or non-identity) is significant and the number of bands should be reported. The demonstration of one or more precipitins indicates infection, including aspergilloma. Precipitating antibodies are often detectable in serum from patients with ABPA. Although one or two precipitins can occur with any clinical form of Aspergillosis, the presence of three or more bands is invariably associated with either an aspergilloma or IA. The test may be negative for some patients receiving long-term antifungal or corticosteroid therapy. When used with reference antisera the ID test is 100% specific (26).

BLASTOMYCOsis:

The ID test for Blastomycosis is positive in approximately 80% of culturally confirmed cases (18). A negative test has little value and in no way excludes the existence of active Blastomycosis. A positive test provides presumptive evidence of active or recent infection. Sera from patients with other mycotic infections (Histoplasmosis, and Coccidioidomycosis) may produce bands against Blastomyces Antigen; however, these bands do not form identity reactions with the "A" band produced by the Blastomyces Positive Control (3). Antibody detection is useful for testing and monitoring patients with suspected blastomycotic meningitis and for assessment of the response to antifungal therapy. The ID test for Blastomycosis with B. dermatitidis "A" antigen is specific. Positive reactions can be the basis for immediate treatment of the patient without the need for parallel tests with Coccidioides and Histoplasma antigens (26). Negative tests, however, do not exclude a diagnosis of Blastomycosis. Only specimens that produce lines of identity or partial identity with the "A" antigen are considered positive for Blastomycosis. The ID test permitted the serodiagnosis of 79% of 113 proven cases of Blastomycosis (26). Sera from some patients with Blastomycosis, however, are not easily found to be positive by the ID test. Patients with negative serum reactions should be studied intensively for culture or histological evidence of Blastomycosis, and several serum samples should be obtained at 3-week intervals and examined for the development of "A" antigen precipitin bands (26). In patients with established cases of Blastomycosis, the disappearance of the "A" antigen precipitin band is evidence of a favorable prognosis (26). The serologic reactivity, however, does not change as rapidly as the clinical response (26).

CANDIDIASIS:

The ID test for detection of antibodies to Candida species is appropriate for sera from patients with candidemia, pneumonitis, endocarditis, wounds, or intra-abdominal abscesses and indwelling urinary or intravascular catheters (26). Debilitated patients and those receiving immunosuppressive agents and prolonged courses of antibiotics are at high risk for invasive candidiasis (26). When they become granulocytopenic and develop an unexplained fever, they should be tested for antibodies to Candida species (26). The detection of precipitins are considered presumptive evidence of systemic candidiasis, but they may also indicate colonization or transient candidemia (26). The ID test for antibodies has a sensitivity of about 80% for the confirmation of invasive candidiasis in immunologically intact hosts (26). Because the Candida antigens of diagnostic significance have not been defined, any precipitin band (whether identity, partial identity, or non-identity) is significant and the number of bands should be reported. The Candida ID Positive control should contain at least two precipitins. The production of one or more precipitins constitutes a positive reaction. Systemic candidiasis should be strongly suspected when serial specimens demonstrate seroconversion (i.e. when negative antibody tests results become positive) or show increases in the number of precipitins (26).
COCCIDIOIDOMYCOSIS: Formation of an "IDCF" and occasionally "IDTP" band(s) between a patient specimen and the Coccidioides Antigen is presumptive evidence of C. immitis infection, current or recent. Some individuals continue to produce detectable antibodies for significant periods (up to 1 year) after clinical recovery from active disease (14). A negative test does not exclude Coccidioidomycosis (3, 14, 17, 23, 28, 29). Cross-reactions may be seen in patients harboring other systemic fungi (especially H. capsulatum) so care must be exercised when reading for identity reactions (3, 17, 29). Latex and complement fixation testing may provide important additional information regarding the patient status (14). Serum precipitins may be detected within 1 to 3 weeks after the onset of primary infections in a large percentage of patients even before complement fixation test (CF) results become positive. In about 80% of all infections, an "IDTP" precipitin is observed within 2 weeks of the onset of symptoms and infrequently is detected 6 months after infection (26). False-positive IDTP reactions have been encountered with samples from cystic fibrosis patients (26). The IDCF test is highly specific. The semi-quantitative IDCF test yields results comparable to those obtained by the CF test (24). The semi-quantitative IDCF titer is not identical to the CF test, but the trends are comparable (24). The semi-quantitative IDCF test on serial specimens may show differences in intensity and banding patterns, which has prognostic value (24, 26).

HISTOPLASMOsis: Serologic evidence is often the prime factor in a definitive diagnosis of Histoplasmosis. The clinically significant antigens of H. capsulatum are designated "H" and "M" antigens. (Note: the "H" band appears closest to the positive control well, whereas, the "M" band is closest to the antigen well.) Precipitins against the "M" antigen are the first to appear in acute pulmonary Histoplasmosis and form the basis of a specific immunodiagnosis. "H" precipitins occur later and less frequently, and their presence is more often linked to extrapulmonary dissemination. The "M" band has been found in about 63% of patients with culture proven Histoplasmosis, while the "H" band is apparent in only 27% (6). The "H" band is rarely observed in the absence of antibodies against the "M" antigen. The "M" band may appear in patients who have recently recovered from Histoplasmosis as well as in the serum of previous Histoplasmosis patients who have recently had a positive skin test (15). Since the test may be negative in as many as 10% of culturally demonstrated cases, the absence of an "H" and/or an "M" band does not rule out Histoplasmosis (6, 13, 15). The combination of ID and CF tests will react with about 85-94% of sera from patients with Histoplasmosis (6, 16). The presence of only "M" antibodies in serum may be attributed to active disease, inactive disease, or skin testing in previously sensitized hosts. The serum from about 70% of patients with proven Histoplasmosis contains "M" precipitins, whereas only 10% of the sera demonstrate both the "M" and "H" precipitins (6). The demonstration of both the "M" and "H" bands is highly suggestive of active Histoplasmosis, regardless of other serologic results. The detection of "M" and "H" precipitins in CSF specimens indicates meningeal Histoplasmosis (26).

PARACOCcidioidomyCOSIS: The ID test using P. brasiliensis antigens has a sensitivity of 94% with sera from patients with paracoccidioidomycosis (26). The main line of precipitation in the ID test shows identity with gp43 (25). Up to three precipitins are observed in sera of patients with paracoccidioidomycosis. A major precipitin, gp43, is closest to the antigen well and is serologically identical to the precipitin lines originally designated "E2" or "A." The precipitin reactive with gp43 is found in 95-98% of patients with seropositive active cases of paracoccidioidomycosis (26). The gp43 precipitin is the most prevalent and is longer lasting than the other two serum precipitins; the latter disappear first in patients with a favorable response to treatment (26). The predictive value of a positive ID result is 100% either at the time of diagnosis or at various periods during and after therapy compared with the results for healthy controls, tuberculosis patients, or those with other mycoses (26).

REFERENCES