ASPERGILLUS SPECIES, ID-*CANDIDA* ANTIBODY SYSTEM (REF CA1001), INDIVIDUAL REAGENTS, AND INDIVIDUAL PLATES 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 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 number, and date for the plate.
4. Record the name, date, and/or lab number of the first patient.
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.
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 band. 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.
4. Record the name, date, and/or lab number of the first patient.
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. 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 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.

INTERPRETATIONS OF RESULTS

Bands of identity or partial identitiy with a Positive Control are considered positive and indicate patient antibody against the antigen in question. Absence of bands or non-indentity 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 SPECIFIC 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 is 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).

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INTERNATIONAL SYMBOL

	Sufficient for "#" Tests	REF	Reference Number
	Expiration Date	LOT	Lot Number
	Manufac-tured by	IVD	In Vitro Diagnostics
2°C-8°C	Storage 2-8°C	CE	Conforms to European Union Requirements

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Rev. #2
Rev. 2020-12-18