

### INTENDED USE

IMMY Immunodiffusion (ID) plates are designed to detect antibodies against *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Histoplasma*, or *Paracoccidioides* in patient serum and positive controls to aid in the diagnosis of each respective disease.

### EXPLANATION

The ID test is a qualitative test employed for the detection of antibodies from patients suspected of having mycoses as visualized by precipitin bands. Additionally, the ID test is a rapid and reliable tool that provides presumptive evidence of infection. Anti-complementary sera in the Complement Fixation (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.

### BIOLOGICAL PRINCIPLES

Immunodiffusion is a qualitative test based on the principles of double diffusion described by Oudin (6) and Ouchterlony (4,5). An antibody and its homologous soluble antigen are placed in separate wells 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. 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 ID plate are arranged to provide each patient test with a known reference so that identity reactions are 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

1. **Cleargel™ 1-Series, 10/pack** (REF CA1019)  
*Note: Not to be used with rabbit antiserum.*
2. **Cleargel™ 4-Series, 6/pack** (REF ID1019)  
*Note: Not to be used with rabbit antiserum.*
3. **Cleargel™ 4-Series, 6/pack – Large well** (REF ID1039)  
*Note: Not to be used with rabbit antiserum.*
4. **Agarose 4-Series, 6/pack** (REF ID1029)

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

All reagents are intended for *in vitro* diagnostic use only!

### 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. 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 (do NOT directly wet ID plate surface).

3. Reading Light: A high-intensity light (VWR Cat# 41447-193 or comparable) is used to read ID reactions.
4. Pipettor and tips: Pipettor and tips capable of delivering 20-35 µl are required for filling 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

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 and crystallization). ID plates must **NEVER** be frozen.

### PRECAUTIONS

All fungal ID plates 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.

### PROCEDURE

1. Label the ID plate with the date and an identifying number, if necessary.
2. Record well contents on the lid of the plate or other alternative method.
3. Using a pipette, fill the positive control wells, #1 and #4 (Figure 1), of the ID plate with the appropriate positive control reagent. *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.*
4. Using a pipette, fill wells #2, 3, 5, and 6 with test samples from patient sera.
5. 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.
6. Fill the center well (#7) with appropriate antigen reagent.
7. Snap the lid of the plate closed, and carefully transfer the plate to a moist, level chamber. Incubate at room temperature for 24 hours.
8. After 24 hours, read and record the ID band reactions with a camera or other alternative method. 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.
9. An **additional** 24 hours is recommended to confirm a negative result. A final report is made after 48 hours total incubation.

### 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 the ID plate 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 band(s) (the reaction between the antigen and positive control reagents) must be present for the patient tests to be valid. If any bands are missing the test should be repeated. 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

bends 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 (REF 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.

### 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 some fungal antigens should make one suspect a positive result. Although a specific diagnosis cannot be made in the absence of identity or partial identity reactions, the number of bands should be reported.

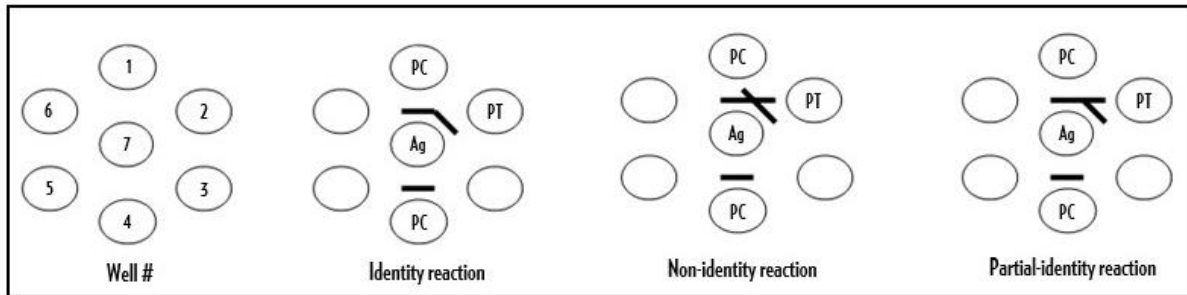
### LIMITATIONS OF THE PROCEDURE

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.

### REFERENCES

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Figure 1.



PC = Positive Control  
 Ag = Antigen  
 PT = Patient Sample

### International Symbol Usage

	Storage 2-8 °C		Lot Number
	Manufactured by		Reference Number
	Expiration Date		In Vitro Diagnostic
	Sufficient for "#" Tests		

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