

**Immunoassay Detection of *Blastomyces dermatitidis* Antibodies Using Lysate
Antigens Prepared from Human, Animal, and Environmental Isolates**

**Kirt A. Jensen, Morgen L. Bybee, James J. Hayden, Summer D. Clark, Sarah J.
Stadelman and Gene M. Scalarone**

Idaho State University, Pocatello, ID 83209

Abstract

Twenty *Blastomyces dermatitidis* yeast phase lysate antigens, prepared from human, animal and environmental isolates, were assayed for sensitivity and specificity with respect to their utility for the diagnosis of blastomycosis. These antigens were used in an indirect enzyme-linked immunosorbent assay (ELISA; peroxidase system) to detect antibodies to *B. dermatitidis* and *Histoplasma capsulatum* in serum specimens from rabbits that were previously immunized with whole killed yeast cells from either organism. The results indicated that the mean absorbance values (sensitivity) ranged from 0.457 to 1.326 when all 20 lysates were tested against the *B. dermatitidis* sera. In contrast, when the lysate antigens were used for antibody detection with the *H. capsulatum* serum specimens the mean absorbance values (specificity) ranged from 0.244 to 0.527. Therefore all of the 20 yeast lysate reagents were able to detect antibody to *B. dermatitidis*, with varying degrees of sensitivity and specificity, but two of the antigens (SOIL, Canada and 397, soil, Georgia) exhibited the greatest potential reactivity at 0.799 and 0.800 respectively. Studies are in progress to further elucidate the potential of the antigens for the clinical diagnosis of blastomycosis.

Introduction

Blastomyces dermatitidis is a dimorphic fungus that causes blastomycosis following inhalation of mycelial conidia into the lungs where the organism converts to yeast cells, causing infection or disease, but the organism may disseminate to other organs including skin, bones, brain, or the urogenital tract [6,7].

For the past several years our laboratory has been performing investigations on the preparation and utilization of yeast phase lysate antigens from different isolates of *B. dermatitidis* for the clinical diagnosis of blastomycosis [1,2,3,4,5].

In this study comparative evaluations were performed using either whole killed yeast cells (antibody induction in rabbits) or yeast lysate antigens (antibody detection in rabbit sera) prepared from 6 different *B. dermatitidis* isolates to determine the reactivity profile of each reagent.

Materials and Methods

Antigens: Antigens were prepared from twenty yeast-phase lysates of *B. dermatitidis* obtained from human, animal, and soil (Table 1). Once mycelial cells had been converted to yeast cells, the cells were grown on a nutritionally lean, chemically defined medium at 37 C for five days with constant shaking.

The cells were collected and washed by centrifugation (5 min at 700 g) with sterile distilled water, repeated five times. Following the wash the cells were lysed by incubation for an additional 7 days at 37 C in sterile distilled water with shaking. Debris was removed from the suspension by centrifugation (30 min at 700 g) and the suspension

sterilized by passage through a 0.2 µm Nalgene filter (Nalge, Rochester, NY).

Merthiolate (1:10,000 dilutions) was added to the antigen preparations as a preservative and the solutions stored at 4 C [8,9,10]. Protein determinations were made for each antigen preparation using bicinchoninic acid (BCA, Pierce, Rockford, IL) method, according to manufacture's specifications.

Indirect ELISA: The ability of the different lysate antigens to detect antibody was evaluated using the indirect enzyme-linked immunosorbent assay (ELISA) utilizing sera that had been collected previously from rabbits immunized with either *B. dermatitidis* or *H. capsulatum* killed whole yeast cells. The indirect ELISA was used for the antibody measurement. Microplates were coated with 100 µL of antigen (100 ng of protein/ml) diluted in carbonate-bicarbonate coating buffer (pH 9.6). The plates were then allowed to incubate in a humid chamber for 24 h at 4 C. Plates were washed three times with 0.15% Tween 20 in phosphate buffered saline (pH 7.4, PBS-T). Diluted serum specimens (100 µl at 1:5000 dilution with PBS-T) were then added to the wells and incubated for 30 min at 37 C in a humid chamber. The plates were again washed as described above. The wells were treated with 100 µl goat anti-rabbit IgG (H&L) horseradish peroxidase conjugate (KPL, Gaithersburg, MD), which was diluted 1:2000 with PBS-T and incubated for 30 min at 37 C. The plates were again washed and 100 µl of horseradish peroxidase substrate was added and the absorbance read at 450 nm using a Bio-Rad model 2550 EIA reader.

Table 1. *Blastomyces dermatitidis* yeast lysate antigens prepared from the following isolates (protein concentrations; $\mu\text{g}/\mu\text{l}$).

(1) T-58 [dog, Tennessee] (80.12)
(2) 48938 [bat lung, India] (143.02)
(3) 394 [soil, Georgia] (75.29)
(4) 591 [human, Eagle River Outbreak] (44.36)
(5) 592 [human, Eagle River Outbreak] (48.67)
(6) 48089 [human, Africa] (17.9)
(7) T-27 [polar bear, Tennessee zoo] (88.35)
(8) ERC-2 [dog, stool, Wisconsin] (44.62)
(9) ER-3 [woodpile, Wisconsin] (68.90)
(10) 643 [human, Oconto Fall Outbreak] (18.12)
(11) 248 [soil, Eagle River outbreak] (23.87)
(12) 86 [soil, Kentucky] (65.76)
(13) 449 [sea lion, Chicago zoo] (58.45)
(14) SOIL [Ontario, Canada-Bakerspigel] (44.10)
(15) 85 [soil, Georgia] (48.53)
(16) 397 [soil, Georgia] (44.10)
(17) B5896 [human, Mountain Iron outbreak, Minn] (49.97)
(18) B5894 [human, Mountain Iron outbreak, Minn] (31.57)
(19) B5929 [human, Mountain Iron outbreak, Minn] (73.47)
(20) KY-H [human, Kentucky] (17.73)

Results

The mean absorbance values of the *B. dermatitidis* (reactivity) and *H. capsulatum* (cross reactivity) antigens are shown in Figure 1. The potential reactivity of the antigens (reactivity minus cross reactivity) is shown in Figure 2.

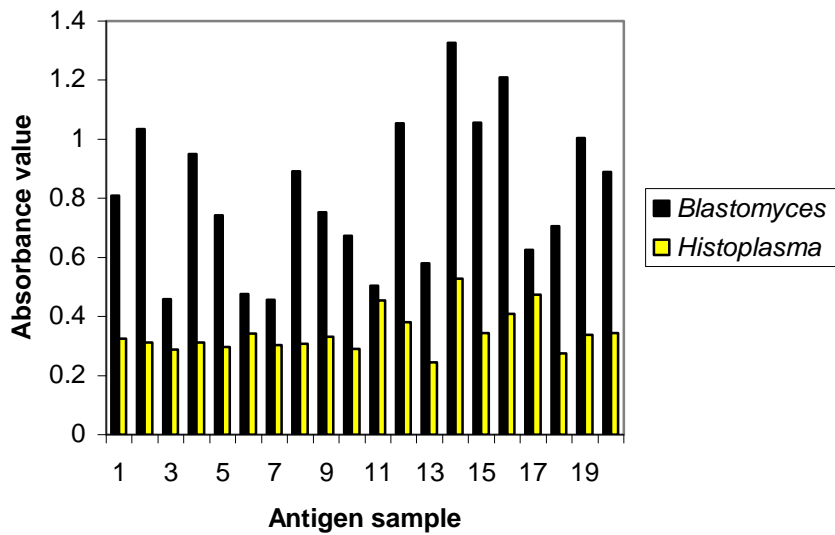


Figure 1. Sensitivity (*B. dermatitidis* sera) and specificity (*H. capsulatum* sera) antibody determinations with the 20 *B. dermatitidis* yeast lysate antigens.

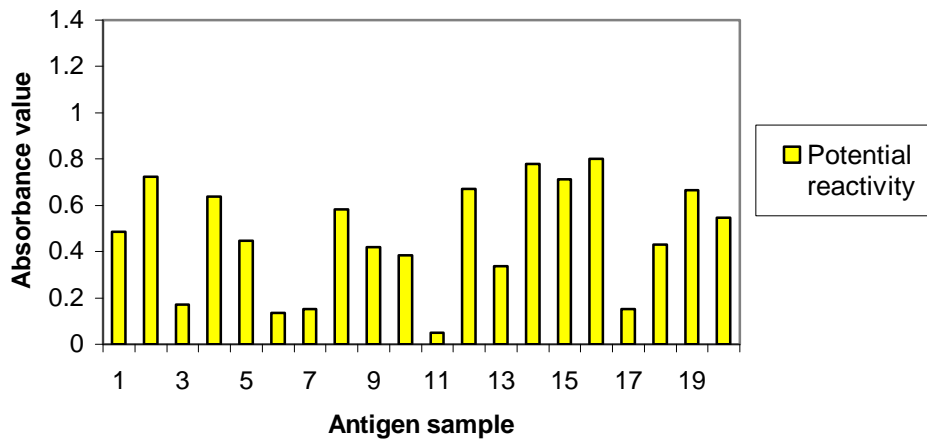


Figure 2. Potential reactivity (reactivity of the 20 yeast lysate antigens with *B. dermatitidis* sera minus the cross-reactivity of the antigens with *H. capsulatum* sera).

Conclusion

A high degree of sensitivity was evidenced with the lysate antigens (absorbance values of 0.457 to 1.326) with minimal cross reactivity (absorbance values of 0.244 to 0.527). The potential reactivity values varied among the 20 antigens, but the SOIL (#14) and the 397, soil, (#16) had the highest values of 0.799 and 0.800. The data indicate the importance of evaluating various antigenic preparations from a variety of *B. dermatitidis* isolates in order to determine what lysate(s) may be appropriate for clinical immunodiagnostic use for the detection of blastomycosis. Additional comparative studies are currently in progress in an attempt to develop a reliable antigenic reagent for diagnostic use.

References

1. Axtell, R.C. & G.M. Scalarone. 2002. Serological differences in two *Blastomyces dermatitidis* isolates from different geographical regions of North America. *Mycopathologia* 153(3):141-4.
2. Axtell, R.C. & G.M. Scalarone. 2002. Serological differences in three *Blastomyces dermatitidis* isolates. *Mycoses* 45(11-12): 437-42.
3. Bakerspigel, A., J. Kane & D. Schaus. 1986. Isolation of *Blastomyces dermatitidis* from an earthen floor in southwestern Ontario, Canada. *J. Clin. Microbiol.* 24:890-891.
4. Baumgardner, D.J. & D.P. Paretsky. 1997. Identification of *Blastomyces dermatitidis* in the stool of a dog with acute pulmonary blastomycosis. *J. Med. Vet. Mycol.* 35:419-421.
5. Baumgardner, D.J. & D.P. Paretsky. 1999. The *in vitro* isolation of *Blastomyces dermatitidis* from a woodpile in north central Wisconsin, USA. *Med. Mycol.* 37:163-168.
6. Davies, S.F. & G.A. Sarosi. 1997. Epidemiological and clinical features of pulmonary blastomycosis. *Semin. Respir. Infect.* 12:206-218.
7. DiSalvo, A.F. 1992. The epidemiology of *Blastomyces dermatitidis*. In: Al-Doory, Y. & A.F. DiSalvo (eds.), *Blastomycosis*. New York: Plenum Publishing Corp, 83-90.
8. Johnson, S.M. & G.M. Scalarone. 1988. Preparation and ELISA evaluation of *Blastomyces dermatitidis* yeast phase lysate antigens. *Diagn. Microbiol. Infect. Dis.* 11:81-86.
9. Levine, H.B., G.M. Scalarone, G.D. Campbell, J.R. Graybill, P.C. Kelly and S.D. Chaparas. 1979. Histoplasmin-CYL, a yeast phase reagent in skin test studies in humans.

Am. Rev. Respir. Dis. 119:629-636.

10. Levine, H.B., G.M. Sclarone & S.D. Chaparas. 1977. Preparation of fungal antigens and vaccines: Studies on *Coccidioides immitis* and *Histoplasma capsulatum*. Contrib. Microbiol. Immunol. 3:106-125.