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Research article

Stability of *Blastomyces dermatitidis* Lysate Antigens Following Prolonged Storage: Sensitivity and Specificity Determinations

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Abstract

The laboratory diagnosis of blastomycosis, caused by the dimorphic fungus *Blastomyces dermatitidis*, has been a diagnostic challenge due to problems with sensitivity and specificity of the assays. The present study assayed 10 lots of *B. dermatitidis* (T-58; Tennessee dog isolate) yeast lysate antigens to determine the stability of the reagents following prolonged storage. The reactivity of the antigens, produced from 1990 to 2012, was determined by comparing antibody detection (enzyme-linked immunosorbent assay; ELISA) with 8 serum specimens from rabbits immunized with *B. dermatitidis* lysate or killed whole yeast cells and 5 serum specimens from rabbits immunized with *Histoplasma capsulatum* yeast lysate antigens. All 10 of the reagents produced during this 22-year period exhibited a high degree of stability and were able to detect antibody in the sera. Sensitivity mean absorbance values ranged from 1.395 (1990) to 1.437 (2012) with a mean value for all 10 antigens equal to 1.355. The mean

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absorbance values for the specificity determinations with the *H. capsulatum* sera ranged from 0.436 to 0.812 with a mean value of 0.504 for all 10 antigens. This study provided evidence that the antigenic reagents do exhibit some lot-to-lot variation in sensitivity and specificity, but they did not lose any appreciable potency during prolonged storage.

Keywords: Blastomycosis, *Blastomyces dermatitidis*, ELISA, lysate antigens, antibody detection

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Introduction

The systemic fungal disease blastomycosis, caused by the etiologic agent *Blastomyces dermatitidis*, is a disease of humans and other animals. Blastomycosis is an endemic disease found in the Southeastern, South-Central and upper Midwestern states of the United States, including areas of Wisconsin, Minnesota and regions of lower Canada. Evidence over the years has indicated that this fungus exists in areas with an abundance of moisture and decaying organic matter [1, 2]. *B. dermatitidis* is thermally dimorphic and acquired by inhalation of the infectious particle (mycelial phase spore) into the lung in which it then converts to a large yeast cell and produces a primary pulmonary acute infection. It may disseminate into other organs of the body including the central nervous system and, as the disease progresses, cutaneous lesions may develop. If a proper diagnosis is not made, or if the disease is misdiagnosed as a bacterial or viral infection, it may be fatal, especially in an immunosuppressed individual [3-6].

Current laboratory diagnostic methods include culturing or histologic identification but, in many instances, these methods may not provide a reliable diagnosis or they may take a considerable amount of time which can delay treatment. During the past several years investigators have made considerable progress with regard to the laboratory diagnosis by developing immunodiagnostic assays for the detection of antibodies or antigens present in patients with blastomycosis [3-7].

In an effort to contribute to improved immunodiagnostic laboratory assays, our laboratory has been concerned with the preparation and comparative studies of *B. dermatitidis* yeast lysate antigens, prepared from various isolates of the fungus, for the detection of antibodies in sera from immunized and infected animals [8-15]. Encouraging results have been obtained with the *B. dermatitidis* lysate antigens, but other studies are needed to further evaluate the reagents with regard to sensitivity and specificity of the reagents prepared from diverse isolates of the fungus obtained from human, animal and environmental sources. Another aspect related to the production and use of these yeast lysate preparations is if these antigens retain their reactivity in antibody detection assays following periods of

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prolonged storage. This characteristic of reagent stability is certainly a desirable attribute if such an antigenic preparation may be developed for commercial diagnostic use. This present study was an extension of a prior study [11] in which not only the sensitivity of 10 lots of *B. dermatitidis* T-58 was evaluated, but also specificity determinations were performed with the antigen preparations following prolonged storage from 2 to 22 years to determine if cross reactivity was evidenced.

Materials and Methods

Lysate antigen preparation

Ten lots of *B. dermatitidis* yeast phase lysate reagents (T-58, dog Tennessee, 1990 to 2012) were prepared by a method similar to one that was previously used for the production of yeast lysate antigen from *Histoplasma capsulatum* [16-18] and modified in our laboratory for *B. dermatitidis* lysate antigen production [8]. The yeast phase cells were grown for 7 days at 37°C in a chemically defined medium in an incubator shaker. They were then harvested by centrifugation (700 x g; 5 min), washed with distilled water, resuspended in distilled water and allowed to lyse for 7 days at 37°C in water with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10,000) and stored at 4°C for up to 22 years. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Thermo-Fisher, Pierce Chemical Company, Rockford, IL) and dilutions of the antigenic reagents used in the ELISA assays were based on protein concentration.

Serum specimens

Eight serum specimens from rabbits immunized with *B. dermatitidis* lysates or killed whole yeast cells and 5 serum specimens from rabbits immunized with *H.capsulatum* yeast lysate antigens were used to evaluate the stored lysate antigens for their ability to detect antibodies in the above sera.

Enzyme-linked immunosorbent assay (ELISA)

The ability of each yeast lysate reagent to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA) as previously described [12-15]. Each lysate antigen was diluted (2000 ng/ml of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and then added to triplicate wells (100 ul) of a NUNC 96-well microplate (Fisher-Thermo). The plates were then incubated overnight at 4°C in a humid chamber followed by washing three times with phosphate buffered saline containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2500 dilution; 100 ul) were added to the microplate wells in triplicate and incubated for

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30 min at 37°C in a humid chamber. Following this incubation, the wells were washed as above and 100 ul of goat anti-rabbit IgG (H & L) peroxidase conjugate (Kirkegaard and Perry, Gaithersburg, MD, KPL) was added to each well and incubated for 30 min at 37°C. The plates were again washed as above and 100 ul of Sure Blue Reserve TMB peroxidase substrate (KPL) was added to each well and incubated for approximately 2 min at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader.

Results

The results as presented in Figure 1 indicate that the *B. dermatitidis* yeast phase lysate antigens did not lose an appreciable amount of sensitivity and the specificity of the reagents was comparable following storage for the 22-year period of storage.

Sensitivity mean absorbance values ranged from 1.395 (1990) to 1.437 (2012) with a mean value for all 10 antigens equal to 1.355. The mean absorbance value difference between the lysates showing the greatest value and the lysate with the lowest value was 0.609. The mean absorbance values for the specificity determinations with the *H. capsulatum* sera ranged from 0.436 to 0.812 with a mean value of 0.504 for all 10 antigens.

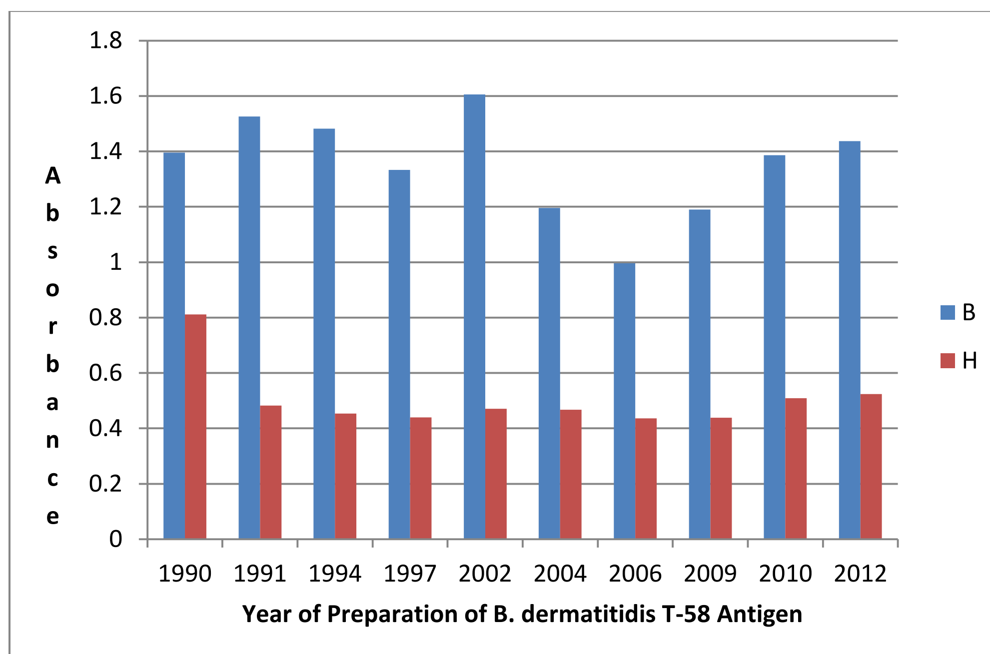

Figure 1. Sensitivity (*B. dermatitidis* antibody: B) and specificity (*H. capsulatum* antibody: H) determinations on 10 yeast lysate antigens (T-58) prepared from 1990 to 2012.

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Discussion/Conclusion

The purpose of this study was to compare the stability of T-58 lysate antigens of *B. dermatitidis* over a 22-year period with regard to antibody detection in serum specimens from rabbits immunized with either *B. dermatitidis* or *H.capsulatum* antigenic reagents. The lysates prepared from 1990 to 2012, and stored at 4°C, exhibited a great deal of stability and able to detect antibody in the *B. dermatitidis* sera. Minimal cross reactivity was evidenced with the stored lysates. Variations in reactivity were observed with the 10 lots of the lysates which were associated with variations in the amount of antibody present in the sera from the rabbits. The stability of immunodiagnostic reagents is certainly an important consideration in the production and use of such preparation for the laboratory diagnosis of infectious diseases. This present evaluation demonstrates that the yeast lysate antigens retained the ability to detect *B. dermatitidis* antibodies in a sensitive manner after storage for a considerable period of time. Studies are continuing in an effort to further evaluate lysate antigens in ELISAs for antibody detection in specimens from animals and humans with blastomycosis.

Acknowledgements

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