US Open Medical Sciences & Medicine Journal
Vol. 2, No. 1, December 2015, pp. 1-7
Available online at http://arepub.com/Journals.php

Research article

Blastomyces dermatitidis Antigen Detection: Comparison of Antibodies Prepared from Lysates and Killed Yeast Cells from a Human and Dog Isolate of the Fungus

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Abstract

Blastomycosis is a difficult disease to diagnose in animals. Recently investigators have concentrated on the development of antigen detection immunoassays for this disease. The current study was designed to evaluate rabbit antibodies produced from *Blastomyces dermatitidis* lysates and killed whole cells from a human and dog isolate of the fungus. The antibodies were used for the detection of antigen in 30 urine specimens from dogs that were known to be infected with blastomycosis. A competitive inhibition enzyme-linked immunosorbent assay (ELISA) was used to compare the antibodies. All four antibodies were able to detect antigen in the urine specimens at different rates with sensitivity values ranging from 46.7% (ERC2 whole cell antibody) to 93.3% (B5931 lysate antibody). When the positive control value of each antibody was compared to the mean inhibition value (the lower the value=more antigen detected) of all 30 specimens, the absorbance value differences between the controls and the urines of each antibody were -0.405 (B5931 lysate), -0.137 (ERC2 lysate), -0.214 (B5931 whole cell) and 0.043 (ERC2 whole cell). The degree of inhibition (antigen detection) was greater with antibodies from both yeast lysate reagents as compared to antibodies produced from killed whole cells.

Keywords: *Blastomyces dermatitidis*, lysate antigens, whole cell antigens, antigen detection, antibody, ELISA, blastomycosis

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Introduction

Blastomycosis, caused by the thermally dimorphic systemic fungal organism *Blastomyces dermatitidis,* is a disease of humans and animals. Blastomycosis is endemic in southeastern regions of the United States as well as in upper midwestern states including Minnesota and Wisconsin and regions of lower Canada. The fungus grows in the mycelial form in areas where there is an abundance of moisture and rich organic matter present [1-3]. Individuals become infected by inhaling the infectious mycelial spore into the lung where the spore may convert into a broad-based budding yeast cell. The disease may present as an acute or chronic infection in the lung or it may disseminate to other internal organs or even to the central nervous system where a fatal meningitis may develop [4-6].

The laboratory diagnosis of blastomycosis has presented a challenge to physicians for many years. Routine microbiological or histological assays may be performed, but in many instances these methods may not yield an accurate diagnosis [3,7,8]. Therefore, in recent years, researchers have devoted a considerable amount of effort in the development of immunodiagnostic assays for the detection of *B. dermatitidis* antibodies or antigens in serum or urine specimens from humans or dogs with blastomycosis or other fungal diseases [7-14].

For several years our laboratory has been involved in the development of *B. dermatitidis* yeast phase lysate antigens from various isolates of the fungus and the evaluation of such lysates for the detection of antibodies in animals. The lysates have also been used to induce antibodies in rabbits and the utilization of such antibodies in antigen detection assays [15-18]. These studies have produced data/results that has been encouraging for continued studies on these reagents as immunodiagnostic tools.

The purpose of this study was to evaluate four separate antibody preparations produced from both lysates and whole cells and to compare them for their ability to detect antigen in urine specimens from dogs that were diagnosed with the disease blastomycosis. The competitive inhibition ELISA was used for the comparative assays.

Materials and Methods

Antigens

Two *B. dermatitidis* yeast phase lysate reagents, from a human outbreak of blastomycosis in Mountain Iron, Minnesota, (B5896 and B5931) and ERC2 (dog isolate, Wisconsin) were prepared by a method similar to one that was previously used for the production of yeast lysate antigen from *Histoplasmacapsulatum* [19-21] and modified in our laboratory for *B. dermatitidis* lysate antigen production [15]. 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 degree C. 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 as immunizing agents and ELISA assays were based on protein concentration.

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Antibodies/Urine Specimens

The four antibodies were obtained from rabbits immunized with either *B. dermatitidis* yeast lysate antigens or with formalin killed whole yeast cell preparations from human isolates B5931and dog isolate ERC2. Urine specimens (30) from dogs diagnosed with blastomycosis were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN)

Competitive ELISA

The horseradish peroxidase competitive binding inhibition ELISA was used for the detection of *B. dermatitidis* antigens in the urine specimens. Microdilution plates (96 well NUNC, Thermo-Fisher) were coated with 100 µl of B5896 (Minnesota human isolate) lysate antigen that was diluted (2000 ng ml-1) in a carbonate-bicarbonate coating buffer (pH 9.6). The plates were 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). Dog urine and 1:1000 (whole cell antibodies) or 1:1250 lysate antibodies obtained from rabbits immunized with either B5931 or ERC2 preparations were added to microcentrifuge tubes (200 µl plus 200 µl of each urine specimen) and incubated for 30 min at 37° C. Following this incubation step 100 µl of the antibody-urine mixture from the microdilution tubes was added to the above plates containing the B5896 antigen and incubated for 30 min at 37º C. The plates were again washed as above and 100µl of goat anti-rabbit IgG horseradish peroxidase conjugate (Kirkegaard and Perry Laboratories, KPL) was added to each well and incubated for 30 min at 37° C and were washed as above. Then 100 µl of Sure Blue Reserve TMB peroxidase substrate (KPL) was added to each well and incubated for approximately 6 min at room temperature. Stop Solution (KPL) was added to each well and the absorbance was read using a BIO-RAD 2550 EIA reader at 450 nm. Positive controls containing known B5896 antigen coated on the plate and the above sera from the immunized rabbits were used to determine the baseline value to which all of the urine specimens were compared.

Results and Conclusion

The four figures (fig. 1, fig. 2, fig. 3 and fig. 4) below represent the data from this experiment. Each of the four antibodies had the ability detect antigen; however, some preparations were found to be more sensitive than others. The absorbance values for the B5931lysate antibody presented a range of 0.689-2.159 with a mean absorbance value of 1.276 while the absorbance values for the ERC2 lysate antibody fell in the range of 0.327-1.669 with a mean absorbance value of 0.609. The B5931 whole cell antibody showed a range of absorbance of 1.183-2.436 and a mean absorbance of 1.687 and the ERC2 whole cell antibody had an absorbance range of 0.522-1.963 with a mean absorbance value of 1.213. The B5931 lysate antibody effectively detected antigen in 28 of the 30 urine samples while the ERC2 lysate antibody did this in 25 of the 30 urine specimens. The B5931 whole cell antibody was efficient at detecting antigen in 24 of the 30 urine samples and the ERC2 whole cell antibody was able to detect antigen in only 14 of the 30 urine specimens.

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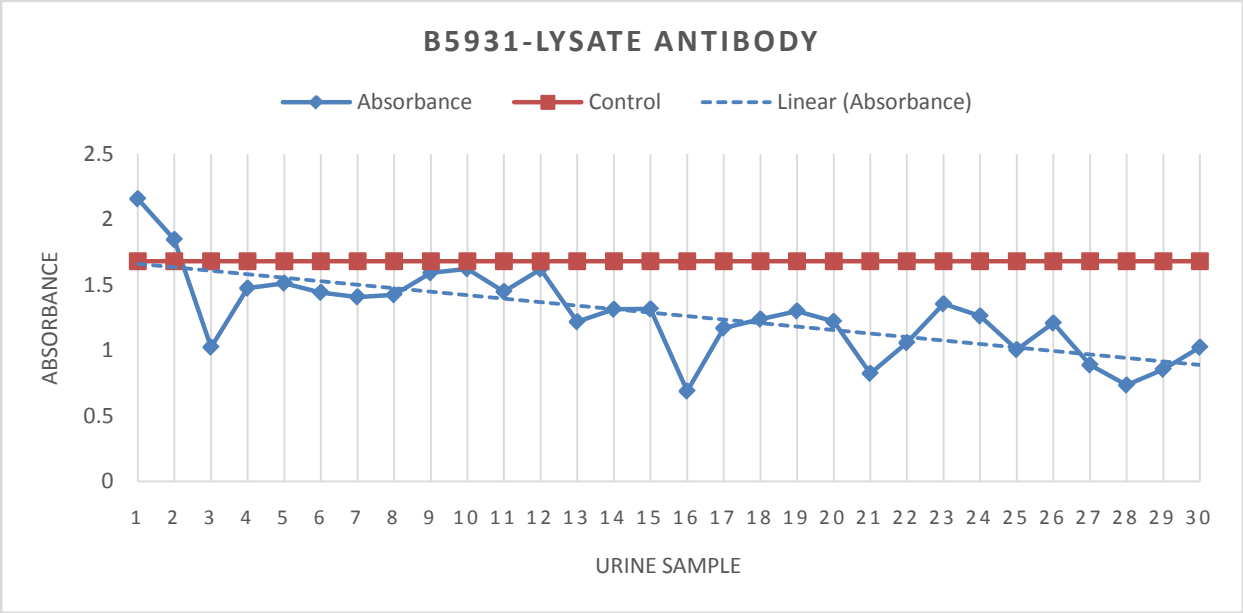

Figure 1. Absorbance values of the B5931lysate antibody against 30 different urine specimens from dogs with blastomycosis.

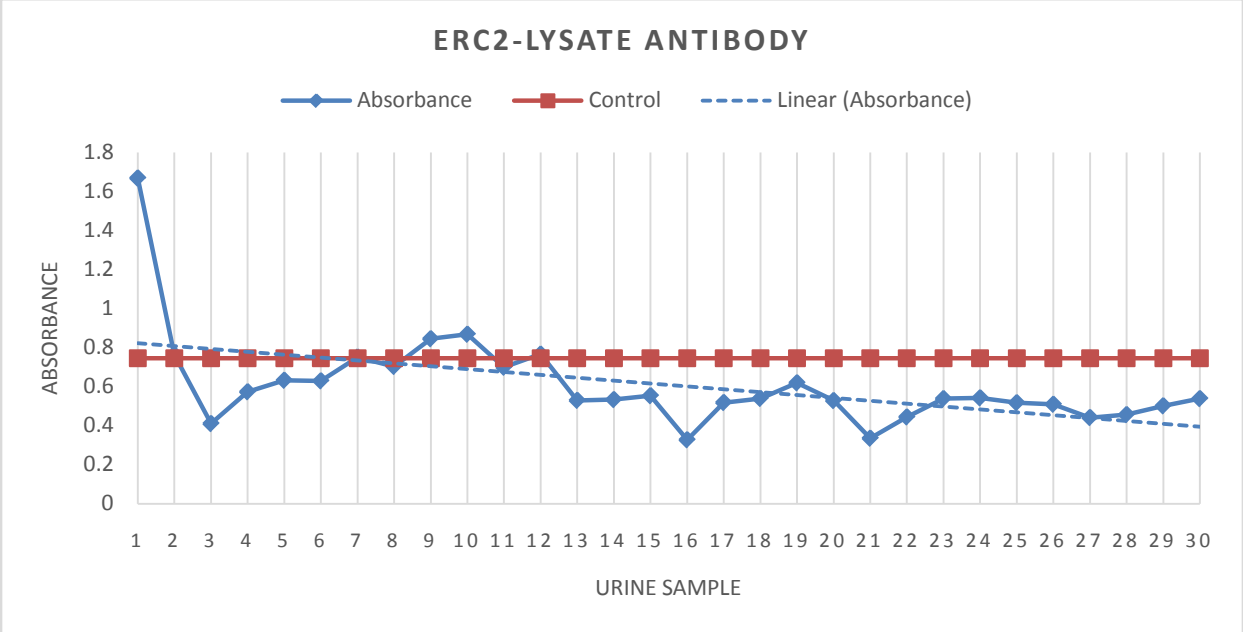

Figure 2. Absorbance values of the ERC2lysateantibody against 30 different urine specimens from dogs with blastomycosis.

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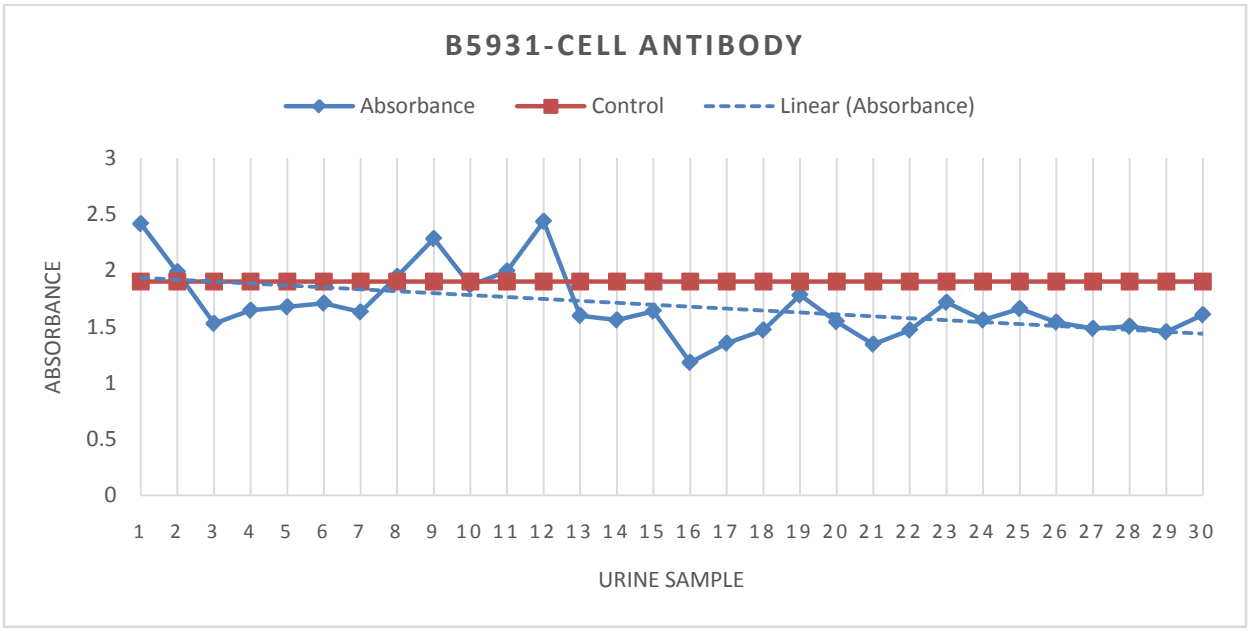

Figure 3. Absorbance values of the B5931 whole cellantibody against 30 different urine specimens from dogs with blastomycosis.

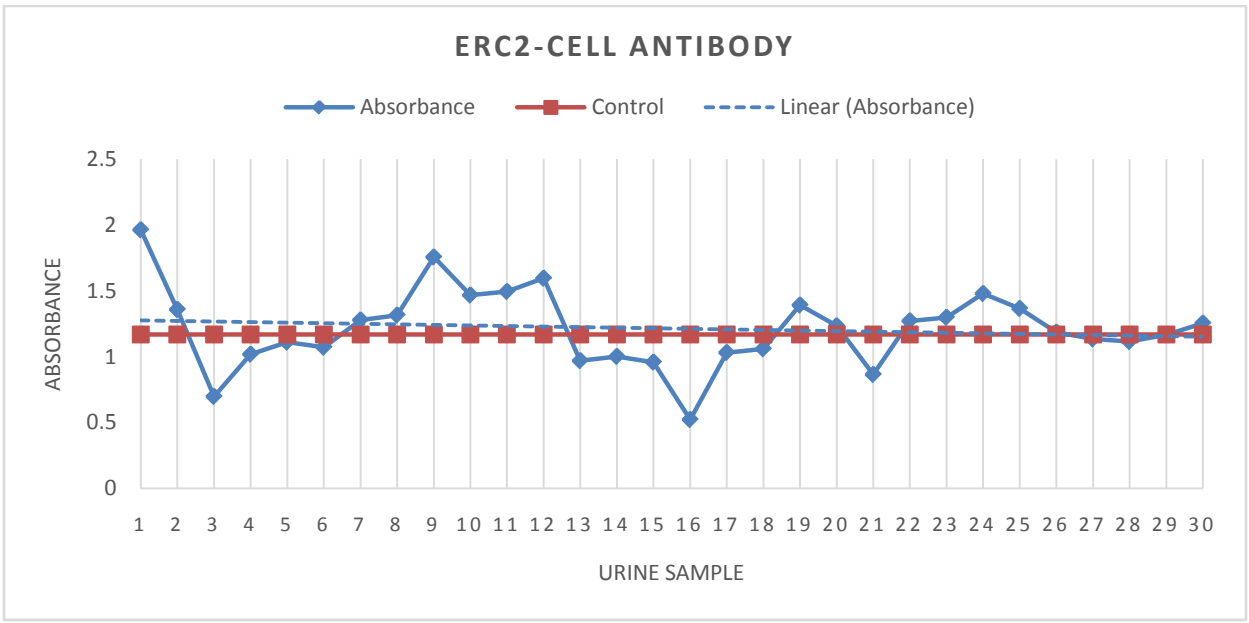

Figure 4. Absorbance values of the ERC2 whole cell antibody against 30 different urine specimens from dogs with blastomycosis.

These results show the differences between the two lysate antibodies and the two whole cell antibodies with regard to antigen detection. The capacity of each of these four antibodies to detect antigen in urine specimens from dogs diagnosed with blastomycosis varied. The B5931 lysate exhibited a 93.3% antigen detection rate while the ERC2 lysate showed an 83.3% detection rate. The study showed that the B5931 lysate antibody was indeed the more effective lysate of the two examined. The B5931 whole cell displayed an antigen detection rate of 80.0% while the ERC2 whole cell fell to only a 46.7% antigen detection rate. The data indicates that the B5931 whole cell antibody is the better one of the two whole cells at detecting antigen in a sensitive fashion. Ultimately, with regard to the four

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antibodies examined in this study, the results of the experiment suggest that the most efficient, most dependable antibody to use when detecting antigen is the B5931 lysate antibody while the least effective antibody at detecting antigen is the ERC2 whole cell antibody. The use of the B5931 lysate antibody would therefore be the optimal reagent when presented with a clinical situation where antigen detection could play a role in discovering an accurate diagnosis.

Acknowledgement

This study was supported by the Department of Biological Sciences at Idaho State University .

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