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**Research article**

# Antigen Detection in Urine Specimens from Dogs with Blastomycosis: Comparison of Rabbit Antibodies Prepared from a Human and Dog Isolate of *Blastomyces dermatitidis*

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## Abstract

Laboratory diagnosis of blastomycosis has relied on antibody detection assays for many years. During the past few years investigators have concentrated on developing improved assays for laboratory detection of antigen for this disease. This present study used a competitive enzyme-linked immunosorbent assay (ELISA) to compare antibodies prepared from killed whole yeast cells of *Blastomyces dermatitidis* isolates *B5896* (Minnesota, human) and ERC-2 (Wisconsin dog) for the detection of antigen in urine specimens from 52 dogs with blastomycosis. The sensitivity ranged from 86.5% with B5896 antibody to 67.3% with the ERC-2 antibody. This comparative assay indicated that the B5896 antibody had a higher degree of sensitivity than ERC-2 antibody and seems to shows potential for the reliable diagnosis of blastomycosis in humans and animals.

**Key words***:* *Blastomyces dermatitidis*, antigen detection, ELISA, dog urine specimens, lysate antigens

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## Introduction

*Blastomyces dermatitidis* is a thermally dimorphic fungus that initially produces a pulmonary disease in humans and animals. It exists as a mold in moist environments and mainly found in states surrounding the Mississippi and Ohio Rivers and the disease is also endemic in Wisconsin and Minnesota plus areas of southern Canada [1-3].

The mycelial phase of the fungus produces microscopic spores or conidia that are the infectious agents that enter the hoist via the respiratory system. In the host the fungus has the unique ability to convert into a budding yeast cell that may remain in the lungs or it may progress to an invasive disease and cause a serious or fatal infection. Dissemination often results in bone, central nervous system and skin involvement [4-7].

The laboratory diagnosis of blastomycosis relies on a combination of clinical history, cytologic/histologic visualization of the yeast cells or by immunodiagnostic methods to detect antibody or antigen in clinical specimens. In certain instances these methods may prove to be inadequate and could result in a non-diagnosis or a misdiagnosis as another microbial disease. Therefore investigators have concentrated on developing improved immuunodiagnostic methods that will provide for rapid and reliable results [6-14]. Antibody detection assays have been faced with sensitivity and specificity problems, but researchers working on antigen detection assays have shown the potential of these assays to provide valuable diagnostic results in both histoplasmosis and blastomycosis [8-14].

Our laboratory has been involved in the utilization of different polyclonal antibodies, produced in rabbits from various *B. dermatitidis* yeast lysates or killed whole yeast cells, for the detection of antigen in urine specimens from dogs with diagnosed blastomycosis [16-18].

Our present project was designed to evaluate and compare antibodies produced in rabbits immunized with *B. dermatitidis* killed whole yeast cells from isolates (B5896, human isolate, Minnesota; ERC-2, dog isolate, Wisconsin) for antigen detection in urine specimens from dogs as above.

## Materials and Methods

***Antibodies***

The antibodies were obtained from rabbits immunized with B5896 (Minnesota, human) and ERC-2 (Wisconsin dog) *B. dermatitidis* killed whole yeast cells. The rabbits were housed in accordance to the NIH guide for Care and Use of Laboratory Animals with approval from the Idaho State University IACUC.

***Urine Specimens***

Fifty- two urine specimens were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN).

***Competitive ELISA method***



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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 1:2200 antibody obtained from rabbits immunized with either B5896 (Minnesota human) or ERC-2 (Wisconsin dog) were added to microcentrifuge tubes (200 µl plus 200 ul of each urine specimen) and incubated for 30 min at 37° C. Following this incubation step 100 ul 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 100 ul 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 BlueTMB peroxidase substrate (KPL) was added to each well and incubated for approximately 2 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/Discussion

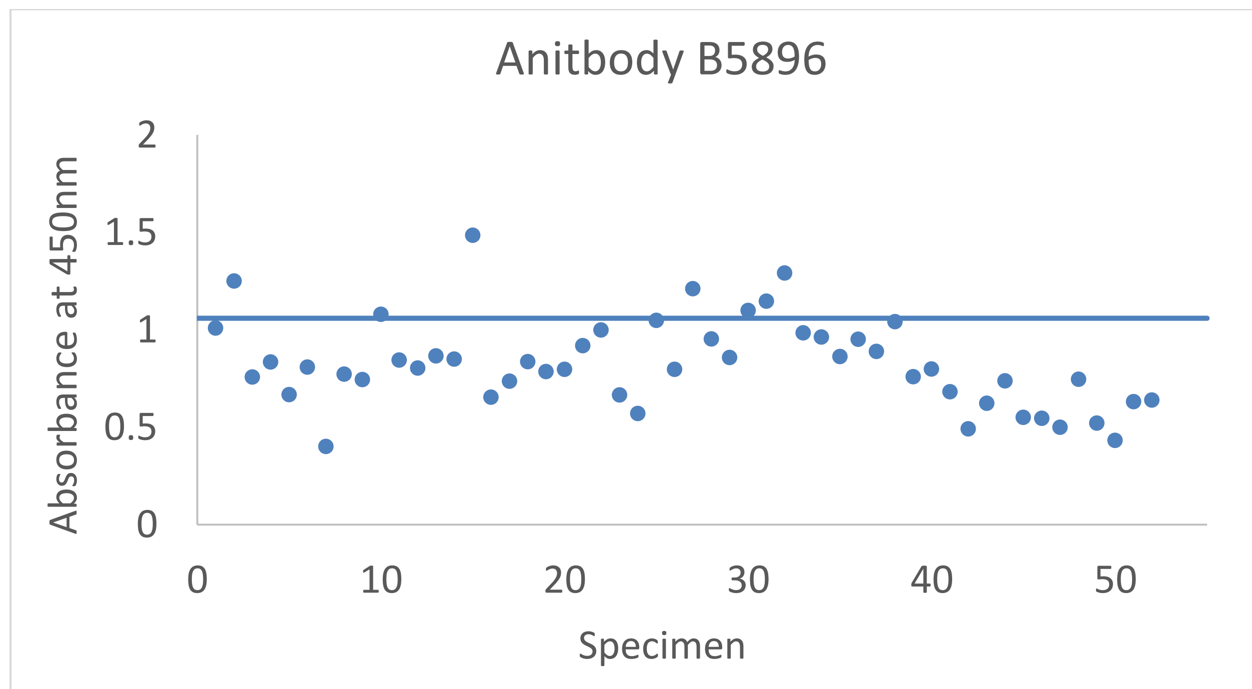


**Figure 1.** The specimens were from 52 dogs that were infected with *B. dermatitidis*. The horizontal line represents the control. Any result that is above the control line shows that no antigen was detected. This figure shows 86.5% antigen detection with the Competitive ELISA.



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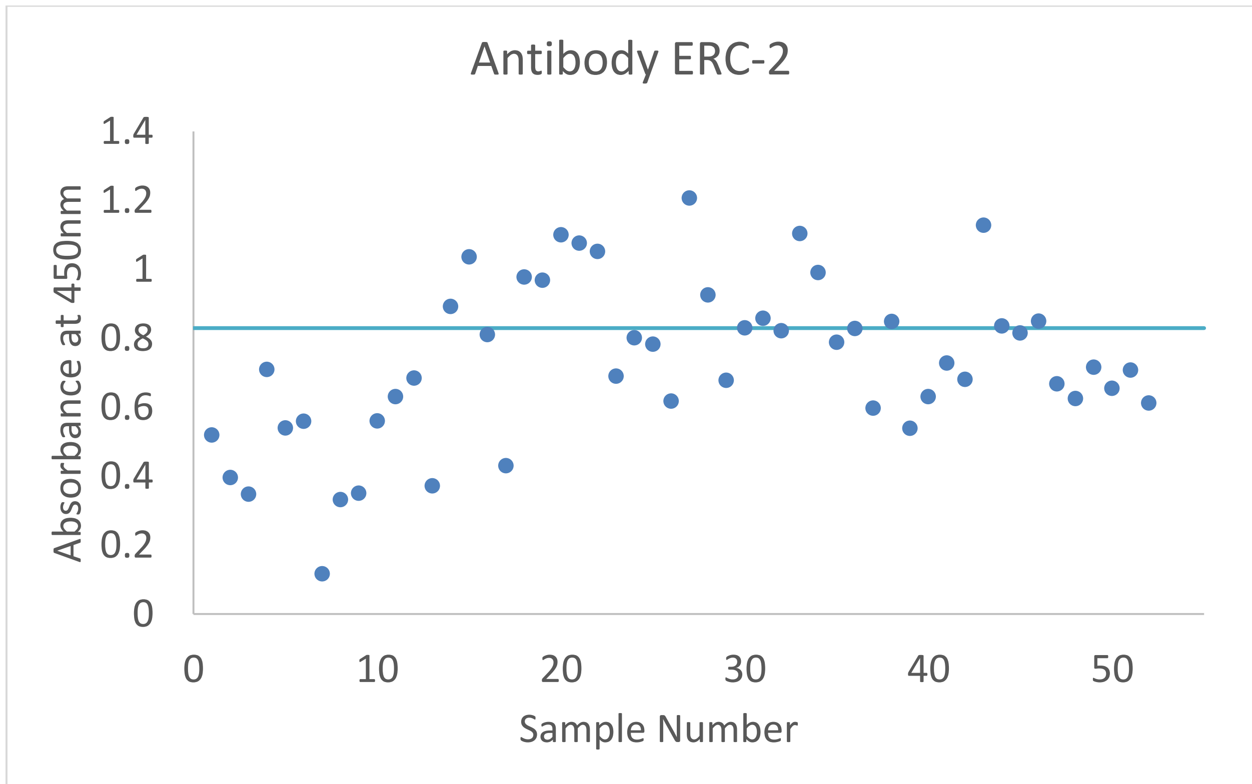


**Figure 2.** The specimens were from 52 dogs that were infected with *B. dermatitidis*. The horizontal line represents the control. Any result that is above the control line shows that no antigen was detected. This figure shows 67.3% antigen detection with the Competitive ELISA.

B5896 antibody detected antigen in 45 out of 52 (86.5%) urine samples from dogs diagnosed with *B. dermatitidis* infections. As Figure 1 shows, the control value was 1.059 and the absorbance value range of antigen detection was 0.4015 – 1.486, values below the control line indicate antigen detection. In contrast the ERC-2 antibody detected antigen in 35 out of 52 (67.3%) specimens. Figure 2 shows the control value was 0.83 and the absorbance value range of antigen detection was 0.1165 – 1.207. Comparing the sensitivity of B5896 antibody and ERC-2 antibody, the B5896 antibody was more effective in detecting *B. dermatitidis* antigen in dog urine than the ERC-2 by 20%.

Many studies have been done pertaining to antibody detection in individuals diagnosed with blastomycosis. This approach has been unsuccessful at diagnosing blastomycosis in immunocompromised individuals because of their inability to produce antibodies. However the detection of antigen presents another option for laboratory diagnostic tests of *B. dermatitidis* infections. Studies are continuing to evaluate antibodies produced by various strains of *B. dermatitidis.*



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