US Open Infectious Diseases & Immunity Journal
Vol. 2, No. 4, November 2015, pp. 1-6
Available online at http://www.arepub.com/Journals.php

Research article

Comparison of Two ELISA Systems for the Detection of *Blastomyces dermatitidis* Antibodies from Immunized Rabbits

Joshua McArthur, Julian Franzen, Tyler James, and Gene M. Scalarone

Idaho State University, 921 South 8th Avenue, Pocatello, ID 83209

Phone: (208) 251-3833 Fax: (208) 282-4570

Corresponding author E-mail Addresses: JM: mcarjosh@isu.edu, GMS: scalgene@isu.edu

Abstract

The diagnosis of blastomycosis is a concern to veterinarians and other health professions in the southern states and along the Mississippi River. Recent research in our laboratory has been associated with the production of *Blastomyces dermatitidis* yeast lysate antigens and the evaluation of these antigens for antibody detection in serum specimens from various sources. The objective of this study was to determine the viability of two conjugate and substrate sources, Kirkegaard and Perry and Santa Cruz Biotechnology for the detection of antibodies in serum specimen from immunized rabbits. It was determined both sources were viable in the detection of antibody from rabbit serum samples. Absorbance values for Trial one using KPL reagents ranged from 0.396 and as high as 2.421, with a mean absorbance value of 1.049. Absorbance values for Trial two using Santa Cruz reagents ranged from 0.288 and as high as 1.168 with a mean absorbance value of 0.632.

Keywords: *Blastomyces dermatitidis*, Antibody Detection, ELISA, Lysate Antigens

Introduction

Endemic to the southeastern United States, *Blastomyces dermatitidis* is an opportunistic fungal pathogen that is of importance to veterinarians. *B. dermatitidis* is a thermally dimorphic fungus with the bulk of its life cycle as a mold

in damp and humid conditions. Given the organism's environmental preferences, the organism has now become endemic and is causing health issues in Wisconsin, Minnesota and along the Mississippi River. Blastomycosis is a respiratory disease caused by *B. dermatitidis* that infects the host through inhalation of the spore, primarily targeting canines and humans. [1-3]

The spore, conidia, is produced by the mycelial phase of the life cycle of *B. dermatitidis*. The mycelial phase life cycle flourishes at 25 ^{0}C as compared to the yeast phase of the organism. The infectious agents enter through the respiratory system and have the ability to convert to the yeast phase of the organisms life cycle, producing budding yeast cells. The yeast phase of the organism grows at an optimal temperature of 37 ^{0}C, giving the organism its pathogenic nature [1-5].

Diagnosis of blastomycosis is difficult in due to clinicians possibly misdiagnosing the respiratory infection as bacterial or viral in nature. In addition, where most of the infected hosts are canine, diagnosis of the disease is not characterized until dissemination to the epidermis occurs. Once in the epidermis the disease becomes prevalent as red lesions spreading across the body [4-9].

The potential misdiagnosis of blastomycosis has driven researchers to develop improved methods of diagnosing this fungal disease. A combination of clinical history, visualization of the yeast cells and/or by immunodiagnostic methods to detect antibody or antigen in clinical specimens is required for a proper laboratory diagnosis of blastomycosis. Reliable methods of diagnosing blastomycosis is paramount to reducing cross-reactivity with *Histoplasma capsulatum.* Inaccurate laboratory conclusions, may lead to an increased probability of the physician prescribing an un-effective plan of treatment. In addition, many physicians may misdiagnose a blastomycosis infection and assume the infecting agent is a bacterial or viral pathogen [8-10].

The immunodiagnostic analysis of blastomycosis has been a continuing focus of research in our laboratory [11-14]. The aim of this study was to determine the viability of two conjugate and substrate solutions from Santa Cruz Biotechnology Inc, and Kirkegaard and Perry.

Materials and Method

Lysate Antigens

The lysate antigens were prepared from a mycelial phase culture of *B. dermatitidis*. Isolate 598, is a human isolate from a blastomycosis outbreak in Eagle River, Wisconsin. The isolate was converted to yeast cells by culturing at 37 ºC on brain heart infusion agar. The yeast phase reagent in this study was prepared by a similar method used in the production of antigen from *H. capsulatum* [15-17] and this method was modified in our laboratory for *B. dermatitidis* lysate antigen production [11]. The yeast cells were grown for seven days at 37 ºC, in a chemically defined medium, in an incubator shaker, and subsequently harvested by centrifugation (700x g; 5 min). The cells were washed with distilled water, re-suspended in distilled water and allowed to lyse for seven days at 37 ºC in water with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10000) and stored at 4 ºC for further use. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Thermo-Fisher Pierce) and dilutions of the antigenic reagent used in the assays were based on the protein concentrations.

Serum Specimens

Twenty five serum specimens from rabbits that were previously immunized with *B. dermatitidis* yeast lysate or whole killed yeast cells were available in our laboratory.

Enzyme-linked immunosorbent assay (ELISA)

The ability of the lysate reagent to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA) in two separate trials. In Trial one, the 598 lysate antigen was diluted (2000ng/mL of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and added to triplicate wells (100µL) of a NUNC 96-well micro plate (Thermo-Fisher). The plates were then incubated overnight at 4 ºC in a humid chamber followed by washing three times with phosphate buffered saline solution containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2000 dilution; 100µL) were added to the micro plate wells and incubated for 30 minutes at 37 ºC in a humid chamber. Following this incubation the wells were washed as above and 100µL of goat anti-rabbit IgG (H & L) peroxidase conjugate, at a dilution of 1:2000 (Kirkegaard and Perry; KPL), was added to each well and incubated for 30 minutes at 37 ºC. The plates were washed again as above and 100µL of Sure Blue Reserve TMB peroxidase substrate (KPL) was added to each well and incubated for approximately two minutes at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance read at 450nm using a BIO-RAD 2550 EIA reader.

Trial 2 was conducted with goat anti-rabbit IgG (HRP) peroxidase conjugate from Santa Cruz Biotechnology at a dilution of 1:1500. The lysate antigen was diluted (2000ng/mL of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and added to triplicate wells (100µL) of a NUNC 96-well micro plate (Thermo-Fisher). The plates were then incubated overnight at 4 ºC in a humid chamber followed by washing three times with phosphate buffered saline solution containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2000 dilution; 100µL) were added to the micro plate wells and incubated for 30 minutes at 37 ºC in a humid chamber. Following this incubation the wells were washed as above and 100µL of goat anti-rabbit IgG (HRP) peroxidase conjugate, at a dilution of 1:1500 (Santa Cruz Biotechnology Inc.), was added to each well and incubated for 30 minutes at 37 ºC. The plates were washed again as above and 100µL of Chem Cruz TMB Substrate Buffer peroxidase substrate(Santa Cruz Biotechnology Inc.) was added and incubated for approximately four minutes at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance read at 450nm using a BIO-RAD 2550 EIA reader.

Results and Discussion

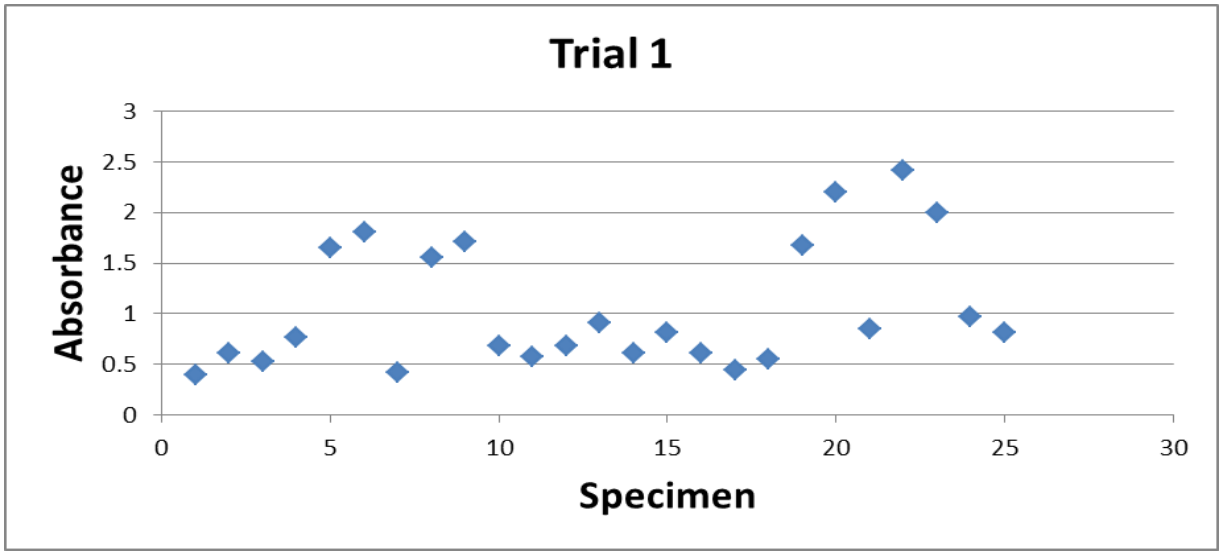

Figure 1: Absorbance values for Trial one using KPL conjugate and substrate solutions.

Figure 1illustrates the absorbance values at 450nm of the 25 serum specimens from rabbits with the KPL reagents. The ELISA test was able to detect antibody in all the samples used in this study at varying levels. A larger absorbance value correlated to a better ability for antibody detection. The 25 serum samples had absorbance values ranging from 0.396 and as high as 2.421, with a mean absorbance value of 1.049.

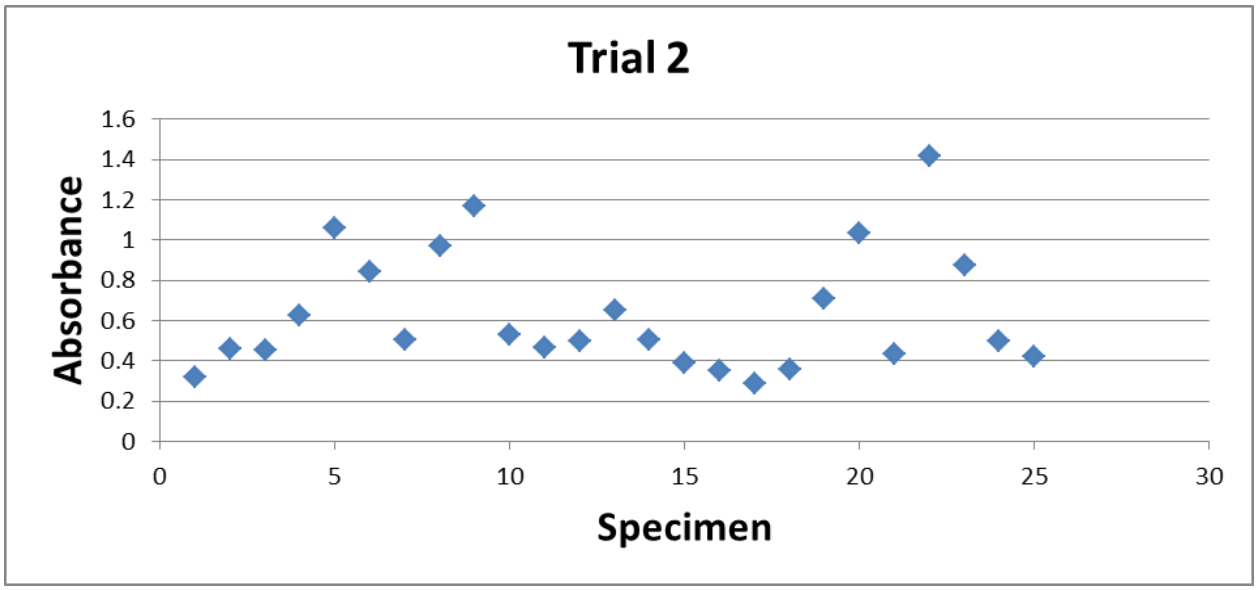

Figure 2: Absorbance for Trial two using Santa Cruz Biotechnology conjugate and substrate solutions.

Figure 2 illustrates the absorbance values at 450nm of the 25 serum specimens from rabbits with the Santa Cruz reagents. The ELISA test was able to detect antibody in all the samples used in this study at varying levels. A larger absorbance value correlated to a better ability for antibody detection. Trial two had absorbance values ranging from 0.288 and as high as 1.168 with a mean absorbance value of 0.632.

Both KPL and Santa Cruz Biotechnology Laboratories' substrate and conjugate products were able to detect antibody present in the serum samples tested. Although both laboratories products are able to detect antibody, the KPL products had a larger mean absorbance value as compared to the Santa Cruz Biotechnology. In addition, the reaction time for the substrate and conjugate to react with each respective company was different. The dilutions for the conjugates utilized for the study, trial one with KPL at a dilution of 1:2000 and trial two with Santa Cruz at a dilution of 1:1500, elicited the optimal results. The reaction time for the KPL conjugate and substrate was approximately two minutes and the Santa Cruz conjugate and substrate required four minutes for optimal reactivity.

Acknowledgement

This research was supported through the Department of Biological Sciences at Idaho State University, Pocatello, ID, USA.

References

[1] A.F. DiSalvo, "Blastomycosis", in *Topley and Wilson's Microbiology and Microbial Infections*," L. Collier,ed., pp. 337-355, Arnold Publishers, London, UK, 9th edition,1998.

[2] M.A. Pfaller and D.J. Diekema, "Epidemiology of invasive mycoses in North America. *Critical Reviews in Microbiology*, vol. 36, no.1, pp. 1-53, 2010.

[3] K. Benedict, M. Roy, T. Chiller and J.P. Davis, "Epidemiologic and ecologic features of blastomycosis: A review," *Curr Fungal Infect Rep*, Vol 6, pp. 327-335, 2012.

[4] M. Saccente and G.L. Woods, "Clinical and Laboratory Update on Blastomycosis," *Clinical Microbiology Reviews*, Vol. 23, No. 2, pp. 367-381, 2010.

[5] R.W.,Bradsher, S.W. Chapman and P.G. Pappas. 2003. "Blastomycosis." *Infectious Disease Clinics of North America*, Vol. 17, pp. 21-40, 2003.

[6] M. Saccente and G.L. Woods, "Clinical and Laboratory Update on Blastomycosis," Clinical Microbiology Reviews, Vol. 23, No. 2, pp. 367-381, 2010.

[7] J.E. Cutler, G.S. Deepe Jr and B.S. Klein, "Advances in Combating Fungal Diseases: Vaccines on the Threshold," Nature Review of Microbiology, Vol. 5, pp.13-18, 2007

[8] M. Saccente and G.L. Woods, "Clinical and Laboratory Update on Blastomycosis," *Clinical Microbiology Reviews*, Vol. 23, No. 2, pp. 367-381, 2010.

[9] J.A. McKinnell and P.G. Pappas, "Blastomycosis: New Insights into Diagnosis, Prevention, and Treatment," *Clinical Chest Medicine*, Vol. 30, pp. 227-239, 2009.

[10] K.S. Vyas, J.R. Bariola and R.W. Bradsher, "Advances in the Serodiagnosis of Blastomycosis," *Current Fungal Infection Reports*, Vol. 2, pp. 227-231, 2008.

[11] S.M. Johnson and G.M. Scalarone, "Preparation and ELISA Evaluation of Blastomyces dermatitidis Yeast Phase Lysate Antigens," Diagostic Microbiology and Infectious Diseases, Vol. 11, pp. 81-86, 1989.

[12] J. Braun, K. Counsell, C. Davis, S. Twamley and G.M. Scalarone, "Antigen Detection in Urine Specimens from Dogs with Blastomycosis: Comparison of Rabbit Antibodies Prepared from a Human and Dog Isolate of Blastomyces dermatitidis," *US Open Infectious Diseases & Immunity Journal*, Vol. 1, No. 3, November 2014, pp. 1 – 6, 2014.

[13] J. B. Poole, R.T. Cox, E.R. Cobb, A.R. Boyd, and G.M. Scalarone, "Comparison of Antibody Detection with Lysate Antigens Prepared from Human and Dog Isolates of Blastomyces dermatitidis," *US Open Infectious Diseases & Immunity Journal*, Vol. 1, No. 1, May 2014, pp. 1 – 5, 2014.

[14] C. Rayner, T. Blackwell, T. Hine, J. Court, and G.M. Scalarone, "A Comparative Study Using 28 Blastomyces dermatitidis Yeast Lysate Antigens for Antibody Detection in Serum Specimens from Immunized Rabbits and Dogs with Blastomycosis," *US Open Infectious Diseases & Immunity Journal*, Vol. 1, No. 2, June 2014, pp. 1 – 8, 2014.

[15] H.B. Levine, G.M. Scalarone, G.D. Campbell, R.C. Graybill and S.D. Chaparas, "Histoplasmin-CYL, A Yeast Phase Reagent in Skin Test Studies in Humans," American Review of Respiratory Diseases, Vol. 119, pp. 629-636, 1979.

[16] G.M.Scalarone, H.B. Levine and S.D. Chaparas, "Delayed Hypersensitivity Responses of Experimental Animals to Histoplasmin from the Yeast and Mycelial Phases of *Histoplasma capsulatum," Infection and Immunity*, Vol. 21, pp. 705-713, 1978.

[17] H.B. Levine, G.M. Scalarone and S.D. Chaparas, "Preparation of Fungal Antigens and Vaccines: Studies on *Coccidioides immitis* and *Histoplasma capsulatum*," *Contributions to Microbiology and Immunology*, Vol. 3, pp. 106-125, 1977.