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Research article

Primary and Secondary Antibody Responses in Immunized Rabbits: Comparison of Induction and Detection with Six *Blastomyces dermatitidis* Yeast Phase Lysate Antigens

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Abstract

Blastomyces dermatitidis is the fungal organism responsible for causing blastomycosis, a disease of humans and animals that is often misdiagnosed and improperly treated. The purpose of this study was to compare antibody induction and detection with 6 *B. dermatitidis* lysate antigens. Indirect ELISAs were performed for antibody detection on sera from immunized rabbits collected on days 14, 28, 42, 49 and 56 (7 days following a booster injection). The secondary antibody responses at 7 days following the booster injection were compared. The greatest mean absorbance values of antibody induction for each detection antigen were as follows: ERC-2 antigen (3.282 with ERC-2 serum), B5896 antigen (2.565 with Cs serum), ER-3 antigen (1.620 with ERC-2 serum), B5931 antigen

(3.364 with ERC-2 serum), Cs antigen (3.314 with ERC-2 serum) and T-58 antigen (3.091 with ERC-2 serum). The B5931 lysate was the optimal detection antigen while the optimal antibody antigen was the ERC-2 preparation.

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

Introduction

Blastomyces dermatitidis, a systemic dimorphic fungus, is the causative agent of blastomycosis, a disease of humans and other animals. It is endemic in the Southeastern and upper Midwestern regions of the US, including highly endemic areas in Wisconsin, Minnesota and in areas of adjoining Canada. *B. dermatitidis* is a thermally dimorphic fungus that exists in regions with considerable organic matter and an abundance of moisture. Blastomycosis is acquired by inhalation of the mycelial spores into the lungs where it then is able to convert into a large broad-based yeast cell [1,2]. Thus a primary pulmonary acute infection is initiated, but the organism also has the ability to disseminate to other internal organs and possibly to the central nervous system with meningeal involvement. In addition cutaneous lesions may develop as the disease progresses. It may be fatal if a proper diagnosis is not made or if the disease is misdiagnosed as a bacterial or viral infection, especially in an immunosuppressed individual [3-6].

With regard to diagnosis, culturing or histologic identification of the organisms have been successful in certain instances, but in some situations these methods may not provide a reliable diagnosis or take a considerable amount of time which may delay treatment. During the past several years investigators have made considerable progress with regard to improved laboratory diagnosis by developing immunodiagnostic assays for the detection of antibodies or antigens in humans and animals with blastomycosis [3-7].

Efforts in our laboratory have 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 [8-16]. Encouraging results have been obtained with the lysate antigens, but additional studies are desired to further evaluate the reagents with regard to the immunodiagnostic potential of the reagents prepared from human, animal and environmental sources. The aim of this present study was to perform an evaluation of yeast

phase lysate antigens, prepared from 6 *B. dermatitidis* isolates, in order to determine the efficacy of the reagents with regard to both induction and detection of antibody in immunized rabbits.

Materials and Method

Lysate antigen preparation

Six *B. dermatitidis* yeast phase lysate reagents, ERC-2 (dog, Wisconsin), B5896 (human, Minnesota), ER-3 (soil, Wisconsin), B5931 (human, Minnesota), Cs (soil, Canada) and T-59 (dog, Tennessee) were prepared by a method similar to one that was previously used for the production of yeast lysate antigen from *Histoplasma capsulatum* [17-19] 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, re-suspended in distilled water and allowed to lyse for 7 days at 37°C with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10,000) and stored at 4 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 in the ELISA were based on protein concentration.

Serum specimens

New Zealand white rabbits were immunized with *B. dermatitidis* yeast lysate antigens (140 ug/ml-protein concentration; 2 ml intramuscular; 1 ml subcutaneous) and serum specimens collected on days 14, 28, 42, 49 following the primary inoculation and 7 days following a post-secondary inoculation on day 49.

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 [8-16]. 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 (1:2000 dilution; 100 ul) were added to the microplate wells In triplicate and incubated for 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-Tek Synergy HT reader.

Results and Discussion

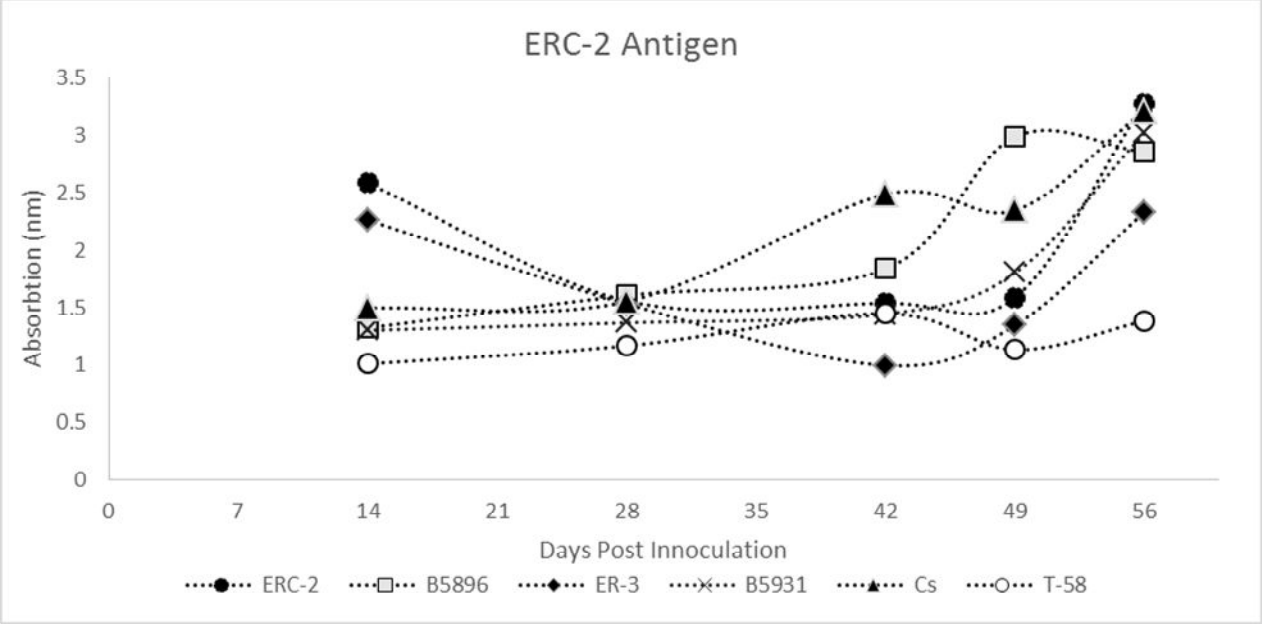

Figure 1: Antibody detection with ERC-2 lysate antigen in serum specimens collected on days 14, 28, 42, 49, and 56 (7 days following a booster injection on day 49) with the 6 antigens (ERC-2, B5896, ER-3, B5931, Cs, and T-58) used to induce the responses.

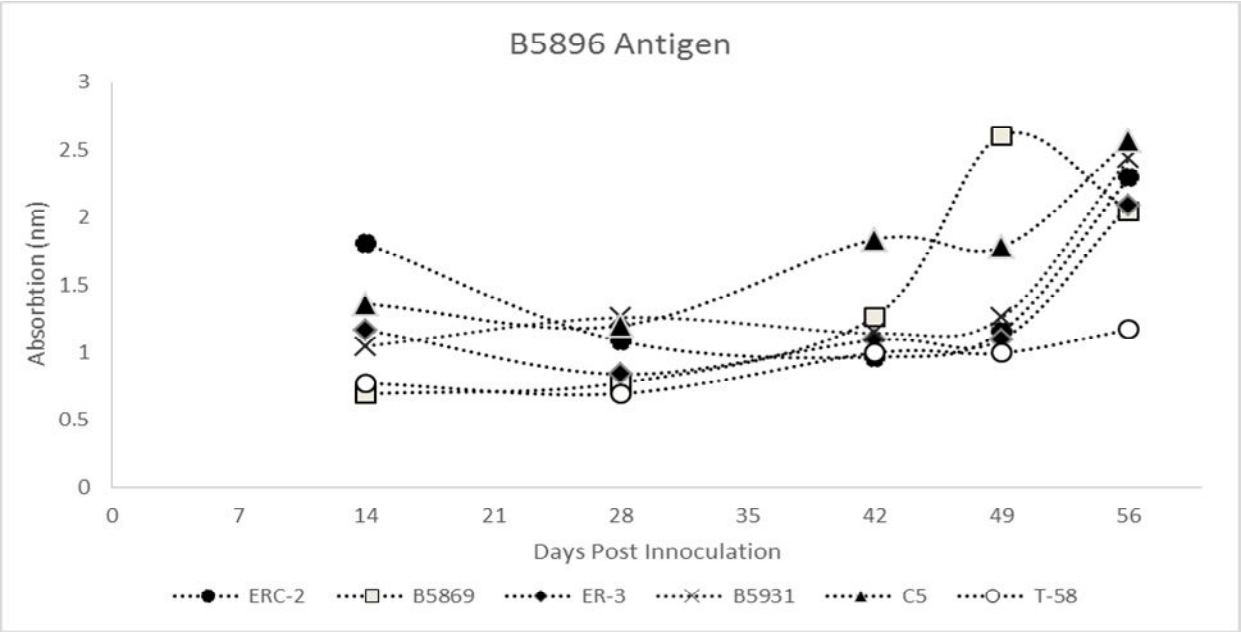

Figure 2: Antibody detection with B5896 lysate antigen in serum specimens collected on days 14, 28, 42, 49, and 56 (7 days following a booster injection on day 49) with the 6 antigens (ERC-2, B5896, ER-3, B5931, Cs, and T-58) used to induce the responses.

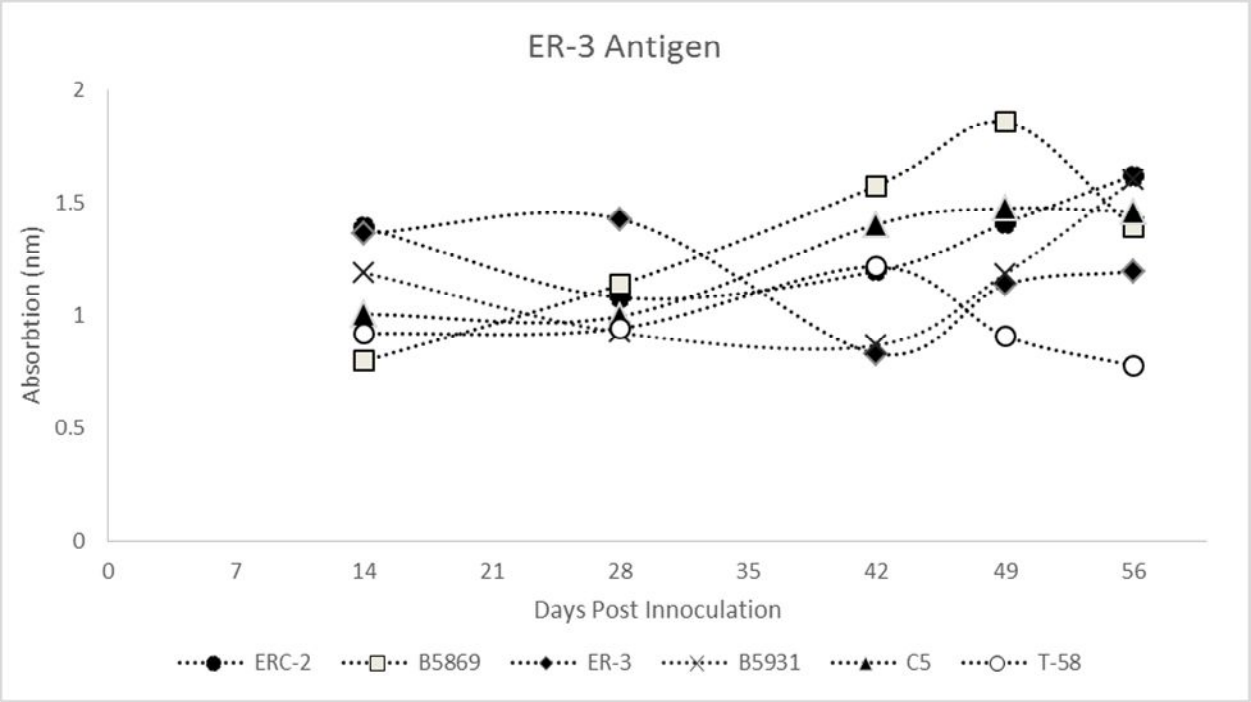

Figure 3: Antibody detection with ER-3 lysate antigen in serum specimens collected on days 14, 28, 42, 49, and 56 (7 days following a booster injection on day 49) with the 6 antigens (ERC-2, B5896, ER-3, B5931, Cs, and T-58) used to induce the responses.

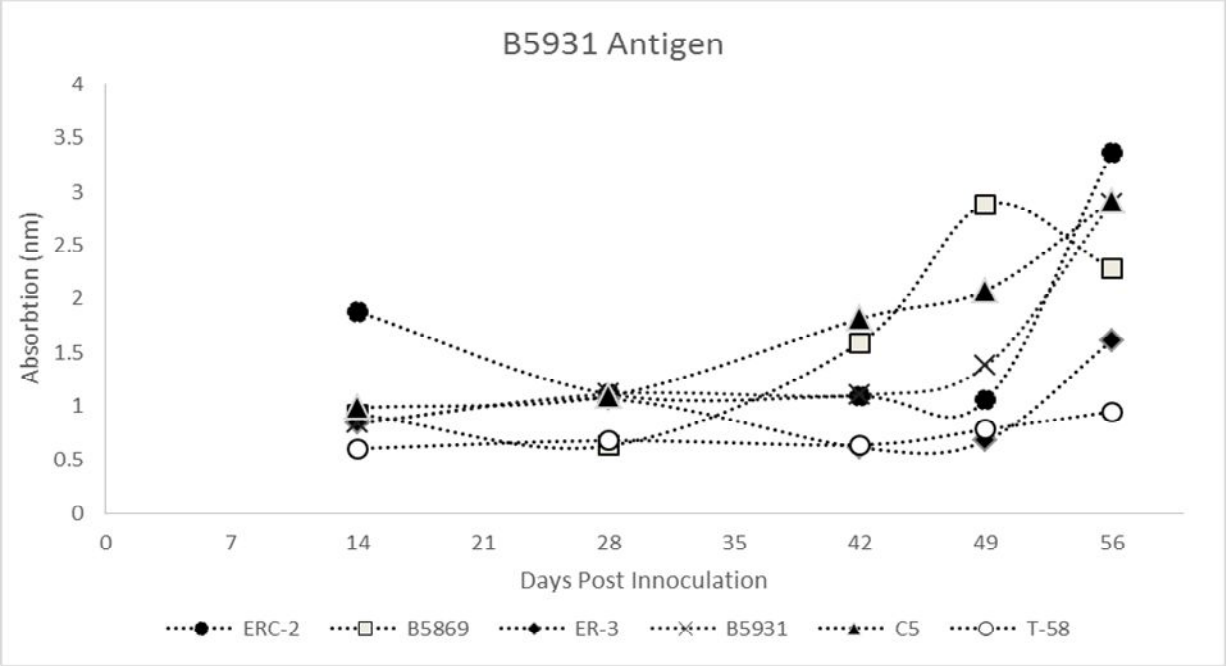

Figure 4: Antibody detection with B5931 lysate antigen in serum specimens collected on days 14, 28, 42, 49, and 56 (7 days following a booster injection on day 49) with the 6 antigens (ERC-2, B5896, ER-3, B5931, Cs, and T-58) used to induce the responses.

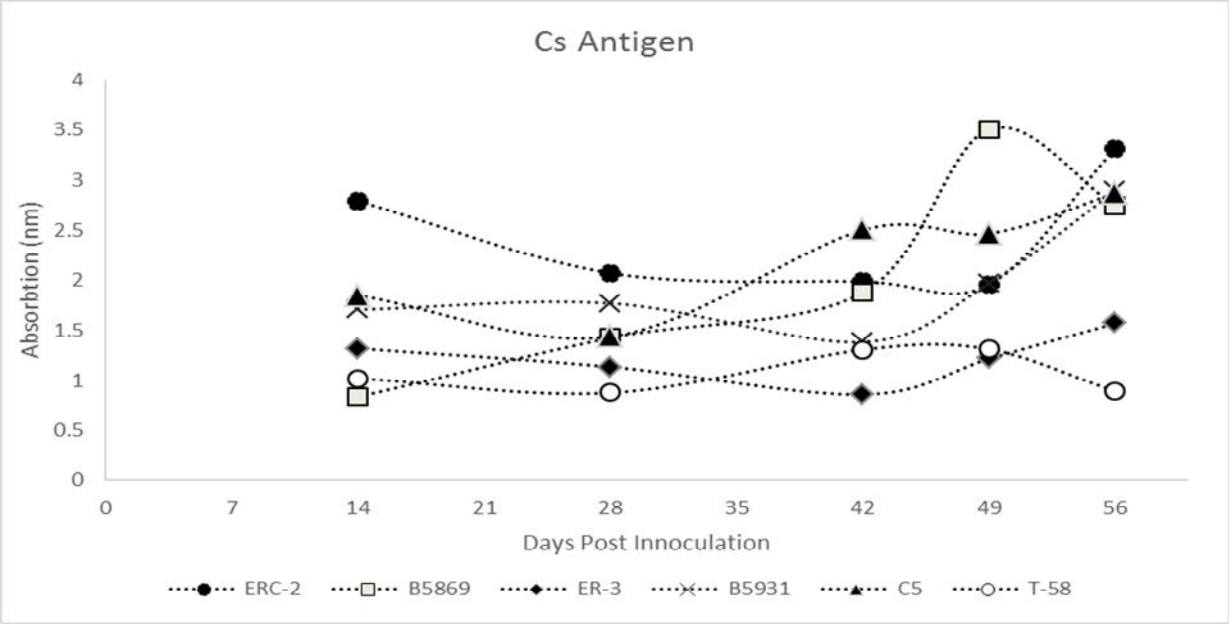

Figure 5: Antibody detection with Cs lysate antigen in serum specimens collected on days 14, 28, 42, 49, and 56 (7 days following a booster injection on day 49) with the 6 antigens (ERC-2, B5896, ER-3, B5931, Cs, and T-58) used to induce the responses.

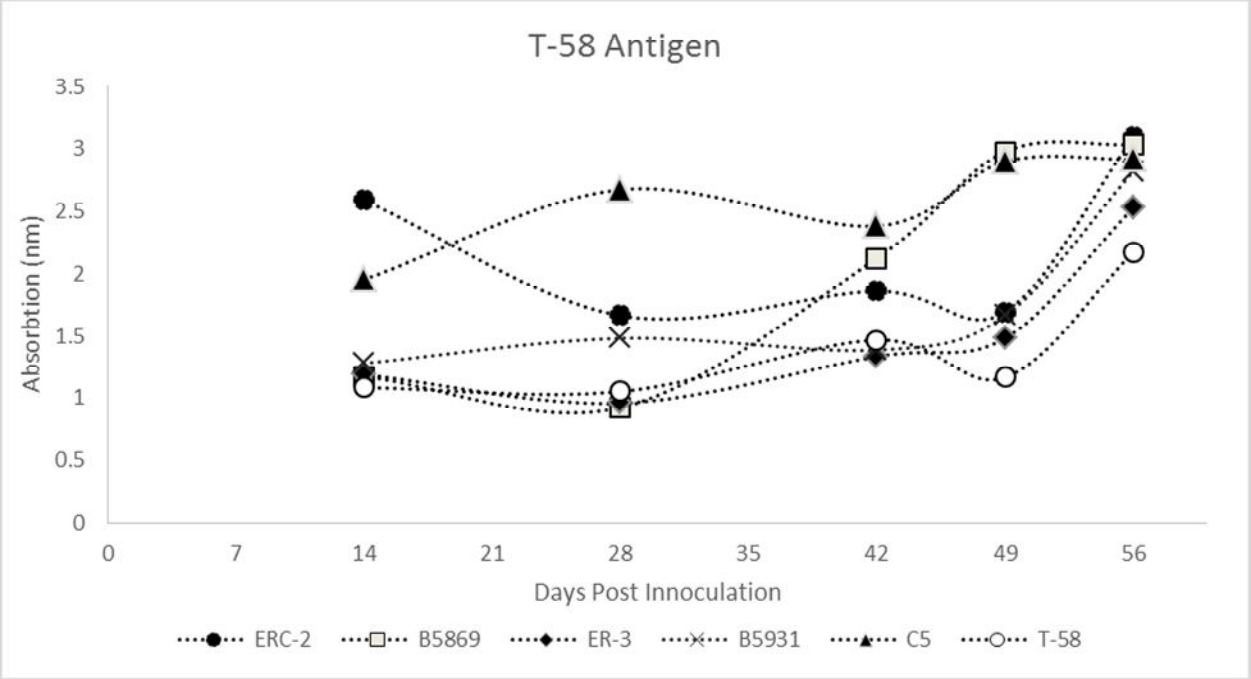

Figure 6: Antibody detection with T-58 lysate antigen in serum specimens collected on days 14, 28, 42, 49, and 56 (7 days following a booster injection on day 49) with the 6 antigens (ERC-2, B5896, ER-3, B5931, Cs, and T-58) used to induce the responses.

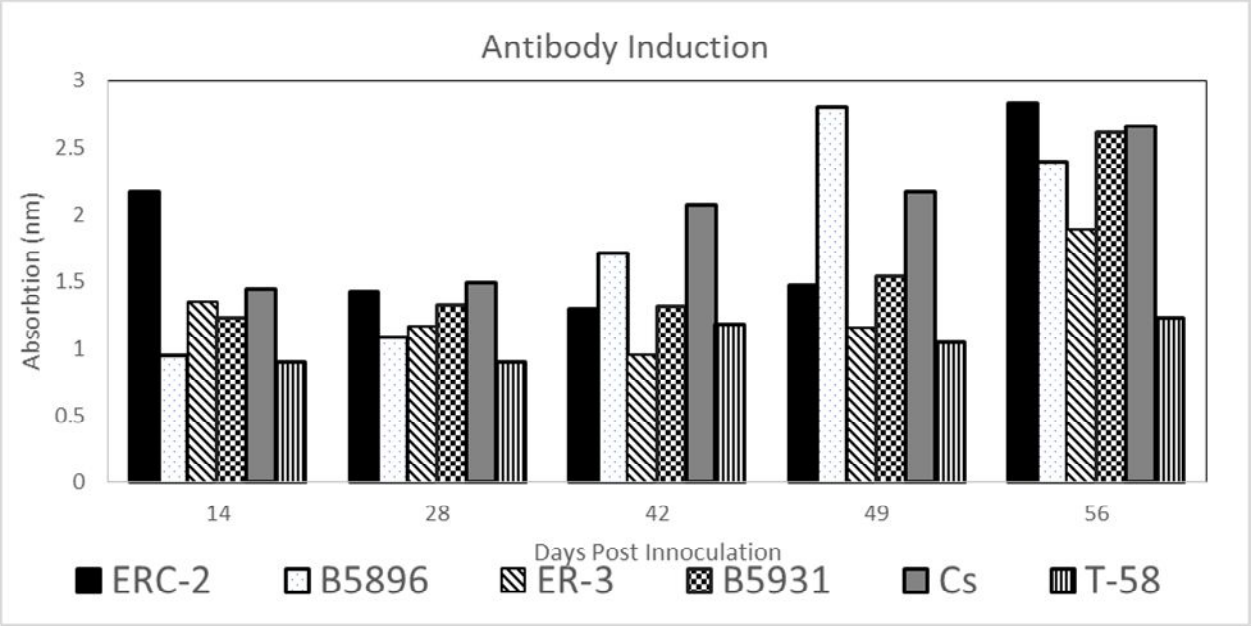

Figure 7: Antibody induction. Figure 7 shows an average of all the 6 serums used with all the antigens.

Figure 1 shows ERC-2 tested against the array of serum specimens. On figure 1, it shows that the ERC-2 serum specimen had the greatest secondary response with a mean absorbance value increase from 1.588nm on day 49 to 3.282nm on day 56. Figure 2 (B5996 antigen) shows that the greatest secondary response was obtained from the B5931 serum specimen. The average absorbance values for B5931 serum specimen increased from 1.259nm at day 49 to 2.432 on day 56. Figure 3 (ER-3 antigen) shows that the greatest secondary response was obtained with B5931 serum specimen which had an increase in average absorbance of 1.181nm on day 49 to 1.599nm on day 56. Figure 4 (B5931 antigen) shows that the greatest secondary response was obtained from the ERC-2 serum specimen with an increase in average absorbance of 1.057nm on day 49 to 3.364nm on day 56. Figure 5 (Cs antigen) shows that the best secondary response was obtained from the ERC-2 serum specimen with an increase in average absorbance from 1.96nm on day 49 to 3.314nm on day 56. Figure 6 (T-58 antigen) shows the best secondary response came from the ERC-2 serum specimen which had an increase of average absorbance from 1.692nm on day 49 to 3.091 on day 56. Figure 7 (Induction) shows the average absorbance of the serum specimens when tested with all the antigens used. ERC-2 had the highest average absorbance on days 14, and 56. Cs had the highest average absorbance on day 28 and 42. And B5896 had the highest average absorbance on day 49.

The data shows that all 6 lysate antigens were able to induce an antibody response and all 6 antigens were able to detect antibody production. The greatest mean absorbance values of antibody induction for each detection antigen were as follows: ERC-2 antigen (3.282 with ERC-2 serum), B5896 antigen (2.565 with Cs serum), ER-3 antigen (1.620 with ERC-2 serum), B5931 antigen (3.364 with ERC-2 serum), Cs antigen (3.314 with ERC-2 serum) and T-58 antigen (3.091 with ERC-2 serum). The B5931 lysate was the optimal detection antigen while the optimal antibody antigen was the ERC-2 preparation.

Conclusion

The data shows that all 6 lysate antigens were able to induce an antibody response and all 6 antigens were able to detect antibody production. The greatest mean absorbance values of antibody induction for each detection antigen were as follows: ERC-2 antigen (3.282 with ERC-2 serum), B5896 antigen (2.565 with Cs serum), ER-3 antigen (1.620 with ERC-2 serum), B5931 antigen (3.364 with ERC-2 serum), Cs antigen (3.314 with ERC-2 serum)

and T-58 antigen (3.091 with ERC-2 serum). The B5931 lysate was the optimal detection antigen while the optimal antibody antigen was the ERC-2 preparation

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