

**A Comparative Evaluation of Two Enzyme-Linked Immunosorbent Assays
(ELISA) for the detection of *Blastomyces dermatitidis* and *Histoplasma capsulatum*
Antibodies**

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Abstract

The objective of this study was to determine the sensitivity and specificity of an indirect peroxidase enzyme-linked immunosorbent assay (ELISA; termed the standard ELISA in our laboratory) when compared to the indirect biotin-streptavidin ELISA (streptavidin-peroxidase ELISA). Sensitivity was evaluated by comparing the level of antibody detection (absorbance value) shown in an indirect ELISA using peroxidase to that seen in an indirect ELISA using biotin and peroxidase-labeled streptavidin by testing 20 *Blastomyces dermatitidis* yeast lysate antigens, prepared from isolates obtained from various geographical locations, against serum specimens collected from rabbits previously immunized with killed whole yeast cells from either *B. dermatitidis* (T-58, 48938, 592, 591, ER-3) or *Histoplasma capsulatum* (G217B). Both ELISA methods were able to detect antibody in the sera, but detection was greater with the indirect biotin-streptavidin ELISA in sera from rabbits immunized with the *B. dermatitidis* antigens (mean absorbance value: 1.009) than in the standard indirect ELISA (mean absorbance value: 0.629). The same trend was observed with the *H. capsulatum* serum specimen (mean absorbance value: 0.404 and 0.257), respectively, but the potential reactivity

(reactivity with the *B. dermatitidis* sera minus the cross-reactivity with the *H. capsulatum* serum) was considerably greater using the biotin-streptavidin assay.

Introduction

Blastomyces dermatitidis is the etiological agent of the systemic fungal disease blastomycosis. The infection results following the inhalation of mycelial spores produced by this thermally dimorphic organism into the lungs where the organism converts into yeast cells. The infection may exist as a primary acute or chronic infection localized in the lungs, or it may also spread to other organs including the skin, bones or central nervous system with a potentially fatal outcome in humans and other animals [3,5]. For the past several years our laboratory has been involved in developing antigenic reagents produced from various isolates of *B. dermatitidis* and in performing investigations on the use of these yeast phase lysate antigens for the detection of antibodies for the laboratory diagnosis of blastomycosis [1,2,4,7]. We have been using the indirect peroxidase ELISA (designated the standard ELISA) in comparative studies on antibody detection, but another more recent adaptation of the basic procedure has been developed.

Immunodiagnostic assays performed in our laboratory and in other laboratories for the detection of *B. dermatitidis* antibodies have relied on the indirect peroxidase ELISA.

This present study was performed in order to assess the potential for increasing the sensitivity of the ELISA with regard to antibody detection with the biotin-streptavidin method. Antibody detection comparative evaluations were performed using 20 *B. dermatitidis* lysate antigens against sera from immunized rabbits to detect sensitivity and specificity of the biotin-streptavidin and indirect peroxidase ELISAs.

Materials and Methods

Antigens: Yeast phase lysate antigens were prepared from twenty *B. dermatitidis* isolates (T-58, dog-Tennessee; 48938, bat lung- India; 394, soil- Georgia; 591, human- Eagle River Outbreak; 592, human- Eagle River Outbreak; 48089, human- Africa; T-27, polar bear- Tennessee zoo; ERC-2, dog stool- Wisconsin; ER-3, woodpile- Wisconsin; 643, human- Oconto Fall Outbreak; 248, soil- Eagle River Outbreak; 86, soil- Kentucky; 449, sea lion- Chicago zoo; SOIL- Ontario, Canada-Bakerspiegel; 85, soil- Georgia; 397, soil- Georgia; B5896, human- Mountain Iron outbreak, Minn; B5894, human- Mountain Iron outbreak, Minn; B5929, human- Mountain Iron outbreak; KY-H, human- Kentucky) by a method similar to one that was previously used for the production of antigen from *H. capsulatum* [6,8] and modified in our laboratory for *B. dermatitidis* lysate antigen production [4]. The yeast phase cells were grown for seven days at 37°C in a chemically defined medium with shaking, harvested by centrifugation, followed by washing with distilled water and then allowed to lyse for seven days at 37°C in water with shaking. The reagent was centrifuged, filter sterilized and stored at 4°C. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Pierce) and dilutions of the antigenic reagents were based on protein concentration.

Serum Specimens: Specimens from rabbits previously immunized with *B. dermatitidis* or *H. capsulatum* killed yeast cells were used in the assays.

ELISA: Antibodies in the above serum specimens were determined using the standard indirect peroxidase ELISA and the biotin-streptavidin peroxidase ELISA as follows:

- (a) Each of the 20 *B. dermatitidis* lysate antigen preparations was diluted (100 ng of protein/ml) in a carbonate-bicarbonate coating buffer and then added to wells (100 µl) of an Immunomaxi 96-well microplate (Phenix Research Products).
- (b) The plates were incubated overnight at 4°C in a humid chamber followed by washing three times with phosphate buffered saline containing Tween 20 (PBS-T).
- (c) The serum specimens (1:5000 dilution) were added to triplicate microplate wells and incubated for 30 minutes at 37°C in a humid chamber.
- (d) The wells were again washed as above and goat anti-rabbit IgG (H&L) peroxidase conjugate (1:2000 dilution) or anti-rabbit IgG (H & L) biotin conjugate (1:4000 dilution) (Kirkegaard and Perry) was added to each well and incubated for 30 minutes at 37°C.
- (e) Additionally, in the biotin-streptavidin assay a streptavidin peroxidase conjugate (1:200 dilution) was added to the anti-rabbit biotin conjugate wells, following the washing step, and incubated as above.
- (f) The plates were washed as above and 100 µl of peroxidase substrate (Ultra TMB; Pierce) was added to each well and incubated for approximately 3 minutes at room temperature, or until solution in wells turned blue.
- (g) The reaction was stopped with the addition of 100 µl of 2N sulfuric acid and the microplates were placed in a BIO-RAD 2550 EIA reader to determine the absorbance reading at 450 nm.

Results

The absorbance values shown in Figures 1-5 demonstrate a comparison of sensitivity and Figure 6 demonstrates a comparison of specificity between the two ELISA methods.

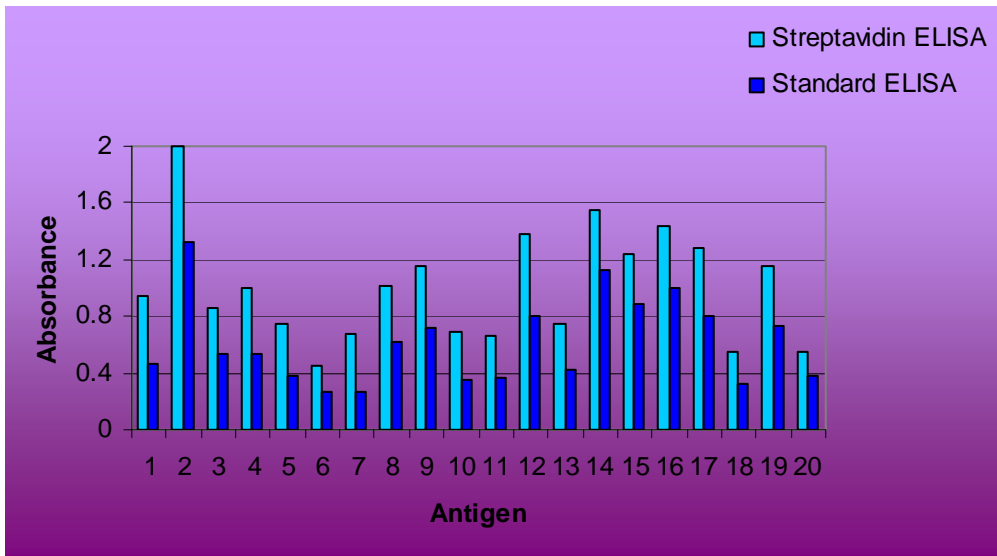


Figure 1: Comparison of the standard ELISA and the biotin-streptavidin ELISA mean absorbance values of each of the 20 *B. dermatitidis* antigens against *B. dermatitidis* serum specimen T-58.

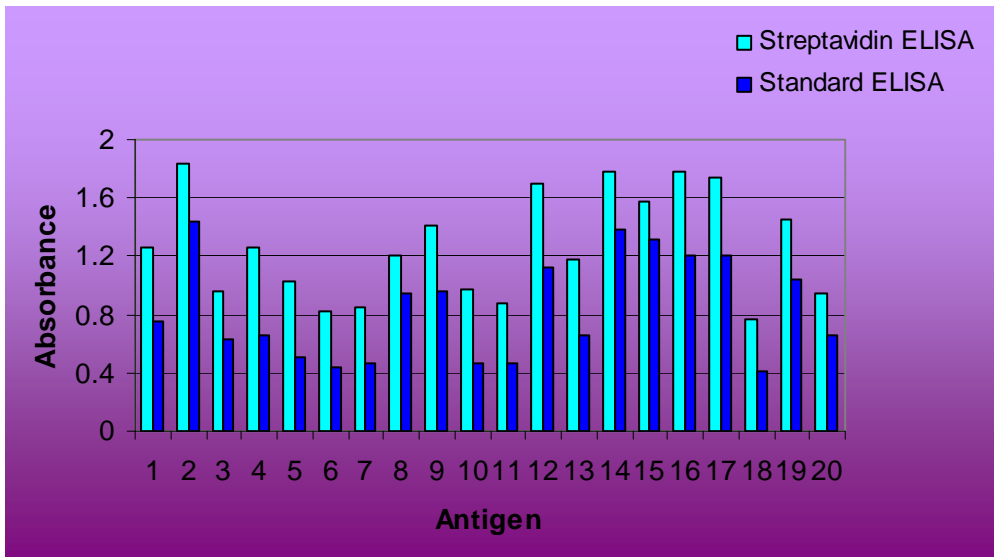


Figure 2: Comparison of the standard ELISA and the biotin-streptavidin ELISA mean absorbance values of each of the 20 *B. dermatitidis* antigens against *B. dermatitidis* serum specimen 48938.

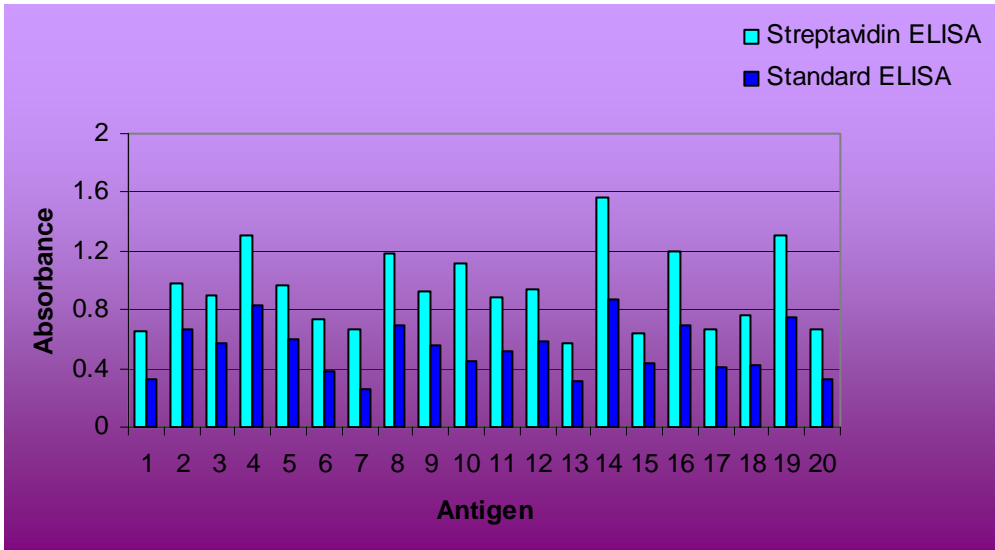


Figure 3: Comparison of the standard ELISA and the biotin-streptavidin ELISA mean absorbance values of each of the 20 *B. dermatitidis* antigens against *B. dermatitidis* serum specimen 592.

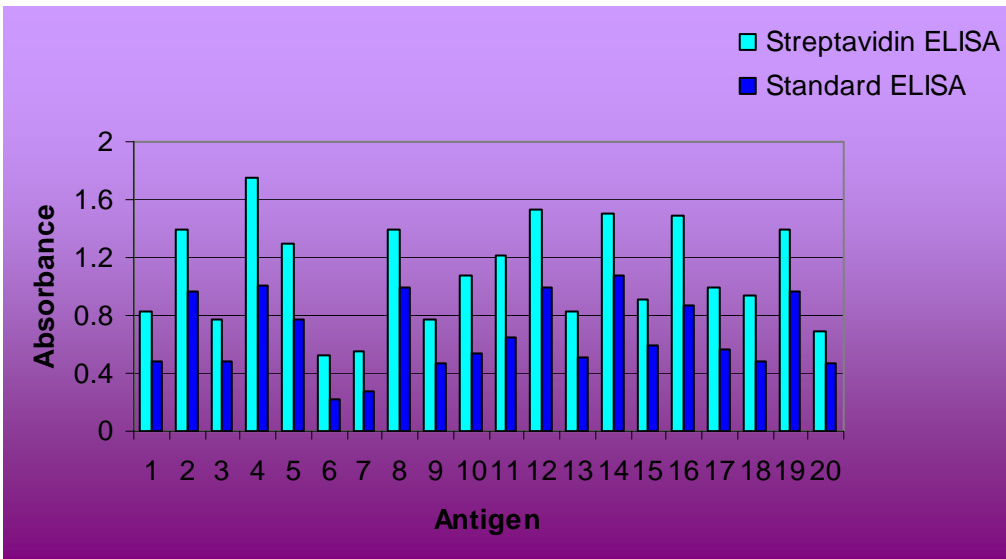


Figure 4: Comparison of the standard ELISA and the biotin-streptavidin ELISA mean absorbance values of each of the 20 *B. dermatitidis* antigens against *B. dermatitidis* serum specimen 591.

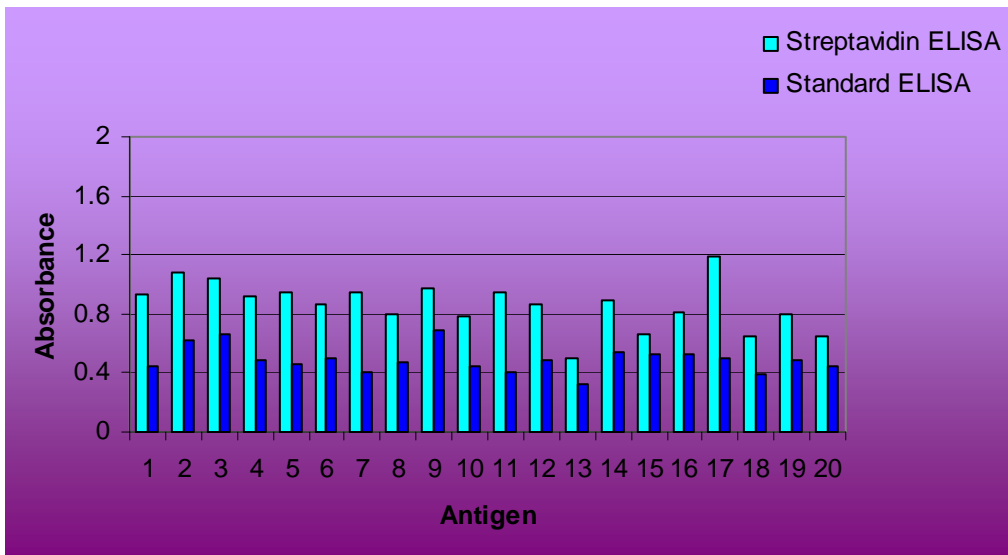


Figure 5: Comparison of the standard ELISA and the biotin-streptavidin ELISA mean absorbance values of each of the 20 *B. dermatitidis* antigens against *B. dermatitidis* serum specimen ER-3.

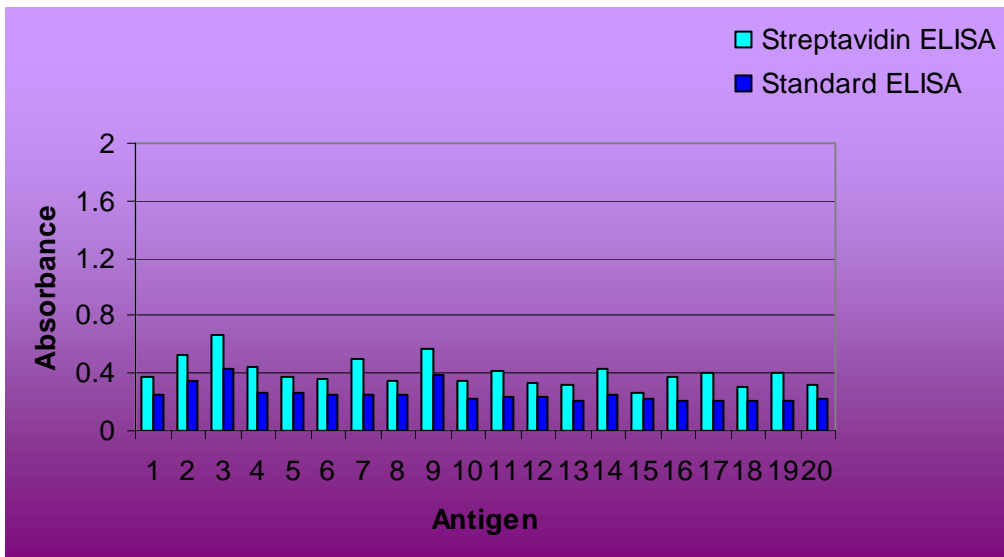


Figure 6: Comparison of the standard ELISA and the biotin-streptavidin ELISA mean absorbance values of each of the 20 *B. dermatitidis* antigens against *H. capsulatum* serum specimen G217B in order to determine the degree of cross-reactivity.

The degree of sensitivity varied among the 20 *B. dermatitidis* yeast lysate antigens depending on the serum specimen assayed. In the sensitivity determinations (Figs. 1-5) the biotin-streptavidin assay exhibited higher absorbance values than the standard peroxidase ELISA with all of the 20 lysate antigens. This pattern of reactivity was also evident when the 20 lysate antigens were used to detect antibody in the *H. capsulatum* specimen (cross-reactivity determination, Fig. 6).

Discussion

Our laboratory has been involved with comparative ELISA studies with various antigenic preparations (yeast phase lysate antigens from different geographical locations in the United States as well as some foreign countries) and different ELISA methods in order to develop the optimal procedure for antibody detection in humans or animals with the systemic fungal disease blastomycosis. Most of our previous studies have been performed using the standard indirect peroxidase method for antibody detection with known antigen, unknown antibody, secondary antibody peroxidase conjugate and substrate (1,2,4,7). The present study was performed in order to compare the standard method with a biotin-streptavidin procedure in order to determine optimal reactivity and specificity parameters. The biotin-streptavidin assay exhibited a greater degree of sensitivity and potential reactivity (reactivity with *B. dermatitidis* serum specimens minus reactivity with *H. capsulatum* sera) as compared to the standard indirect ELISA. This biotin-avidin method offers an alternative to the standard peroxidase method for antibody detection in blastomycosis and may prove to be the method of choice for antibody detection in blastomycosis.

References

1. Axtell, R.C. & G.M. Scalapone. 2002. Seriological differences in three *Blastomyces dermatitidis* strains. *Mycoses*. 45:437-442.
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8. Scalapone, G.M., H.B. Levine & S.D. Chaparas. 1978. Delayed hypersensitivity response of experimental animals to histoplasmin from the yeast and mycelial phases of *Histoplasma capsulatum*. *Infect. Immun.* 21:705-713.

Figure 1: Comparison of the standard ELISA and the biotin-streptavidin ELISA absorbance of each of the twenty antigens against serum specimen T-58.

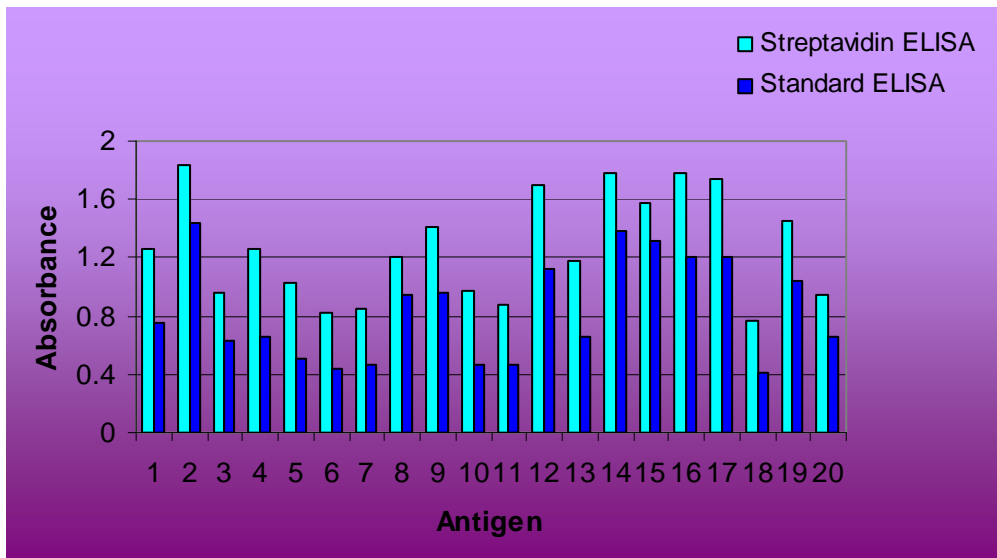


Figure 2: Comparison of the standard ELISA and the biotin-streptavidin ELISA absorbance of each of the twenty antigens against serum specimen 48938.

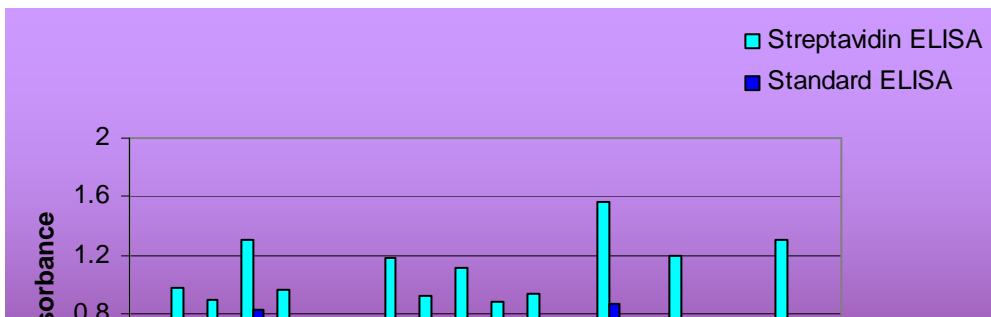


Figure 3: Comparison of the standard ELISA and the biotin-streptavidin ELISA absorbance of each of the twenty antigens against serum specimen 592.

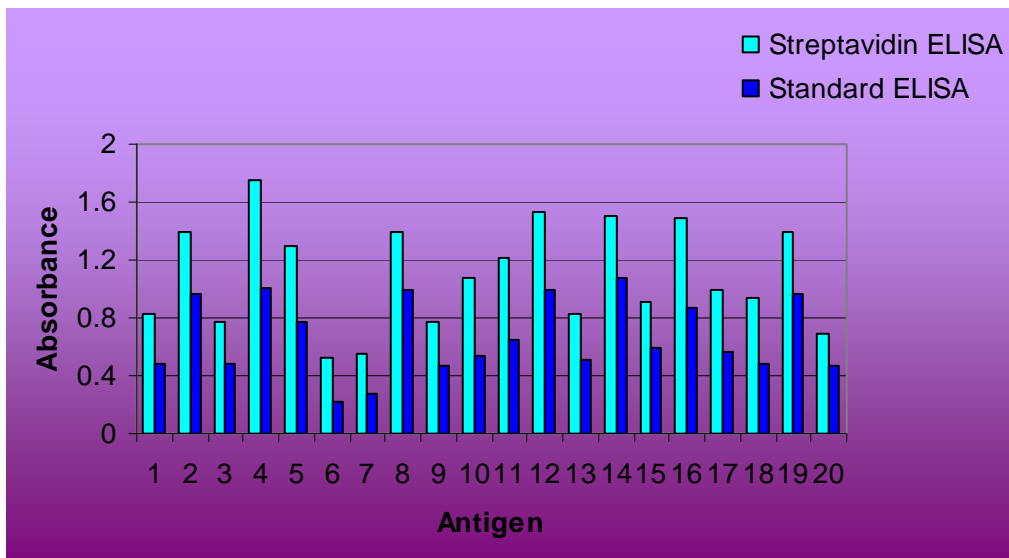


Figure 4: Comparison of the standard ELISA and the biotin-streptavidin ELISA absorbance of each of the twenty antigens against serum specimen 591.

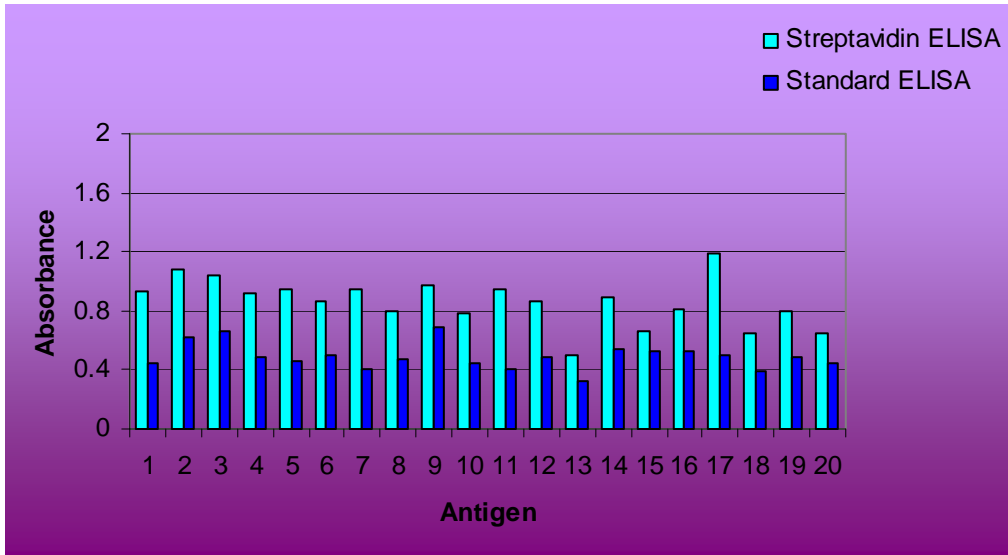


Figure 5: Comparison of the standard ELISA and the biotin-streptavidin ELISA absorbance of each of the twenty antigens against serum specimen ER-3.

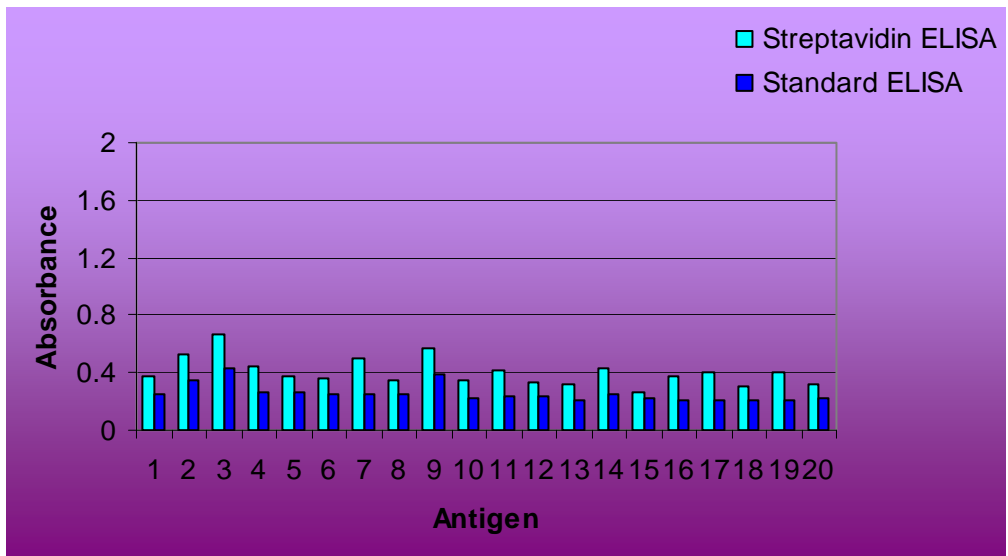


Fig 6: Comparison of the standard ELISA and the biotin-streptavidin ELISA. Absorbance of each of the twenty antigens against serum specimen 6217B.

Discussion

Our laboratory has been involved with comparative ELISA studies with various antigenic preparations (yeast phase lysate antigens from different geographical locations in the United States as well as some foreign countries) and different ELISA methods in order to develop the optimal procedure for antibody detection in humans or animals with the systemic fungal disease blastomycosis. Most of our previous studies have been performed using the standard indirect peroxidase method for antibody detection with known antigen, unknown antibody, secondary antibody peroxidase conjugate and substrate (1,2,4,7). The present study was performed in order to compare the standard method with a biotin-streptavidin procedure in order to determine optimal reactivity and specificity parameters. The biotin-streptavidin assay exhibited a greater degree of sensitivity and potential reactivity (reactivity with *B. dermatitidis* serum specimens minus reactivity with *H. capsulatum* sera) as compared to the standard indirect ELISA. This biotin-avidin method offers an alternative to the standard peroxidase method for antibody detection in blastomycosis.

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