Onchocerca Diagnostic Testing of Polymorphisms; Effects of Antigenic Mutations on Detection of Antibodies by OV-16 ELISA

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ABSTRACT

Onchocerca **is a filarial parasite transmitted to humans by the female blackfly of the *Simulium* genus that is known to cause "river blindness" among humans in regions of Africa and the Americas. ELISA (Enzyme-linked Immunosorbent Assay) serological testing for the presence of antibodies to a diagnostic antigen (Ov16) is a diagnostic tool in current use for verifying suppression of transmission of the parasite. Recently a humanized monoclonal antibody has been developed to use as a positive control in the Ov16 ELISA. This was done as it is becoming harder to obtain positive samples for use as pooled sera for a source of positive controls as elimination grows closer. Recently we have discovered that naturally occurring polymorphisms in the Ov16 antigen affect the binding of this monoclonal antibody. Here, we have identified the particular polymorphism responsible for the loss of monoclonal antibody reactivity and investigated the potential effect these polymorphisms may have on the sensitivity of the Ov16 ELISA for the detection of parasite exposure.**

INTRODUCTION

Onchocerciasis, or river blindness is one of the neglected tropical diseases of mankind. It is also one of the few such diseases targeted for elimination by the international community. This effort is primarily based on a strategy of once or twice per year mass distribution of the anti-helminthic ivermectin to the afflicted communities. This strategy has been successful in eliminating onchocerciasis in all but one focus in Latin America and has also been successful in multiple foci in Africa. Demonstrating transmission has been suppressed, currently relies on entomological assays and detection of exposure to the parasite in children. This is assessed by ELISA detecting IgG4 antibodies against Ov16, a 16kDa immunodominant antigen. However, polymorphisms in this antigen may affect the ability of these antibodies to recognize the antigen, thereby affecting the sensitivity of the ELISA assay. This possibility was found to be more than hypothetical when we found that a positive control humanized monoclonal antibody produced as a positive control for the Ov16 ELISA reacted to a recombinant version of the Ov16 antigen derived from parasites from Guatemala but did not react to a homologue derived from a sequence obtained from parasites from Cameroon. The purpose of this study was to identify the mutation responsible for the loss in monoclonal activity and to determine if the polymorphisms affected the sensitivity of the Ov16 ELISA.

METHODS

- **Two distinct mutations were discovered in the OV-16 antigen occurring at amino acids 167 and 196 when comparing sequence of antigens derived from Guatemala and Cameroon**
- **Clones were produced by in vitro mutagenesis containing no mutations, each of the mutations individually and both mutations.**
- **Each of the Mutated Ov-16 antigens was expressed with a GST tag and purified using affinity chromatography.**
- **SDS gel electrophoresis performed to evaluate antigen purity.**
- **The positive control monoclonal antibody and patient serum samples (n=414) were then tested by ELISA against the four recombinant antigens. Patient sera were drawn from a serum bank collected in the 1980s in Liberia. All individuals in the bank were infected with *O. volvulus*.**
- **Serum samples were from pre-existing samples for which no personal identifiers were available. As a result, the USF IRB ruled on 9/16/16 that this work did not meet the definition of human subjects research.**

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Cameroon    VKNQPTKVSWDAEPGALYTLVMTDPDAPSRKNPVFREWHHWLIINISGQN 151
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Guatemala   VKNQPTKVSWDAEPGALYTLVMTDPDAPSRKNPVFREWHHWLIINISGQN 151

                          *                                  *
Cameroon    VSSGTVLSDYIGSGPFKGTGLHRYVFLVYKQPGSITDTQHGGNRFNFKVM 201
            |||||||||||||||| |||||||||||||||||||||||||||| |||||
Guatemala   VSSGTVLSDYIGSGPRKGTGLHRYVFLVYKQPGSITDTQHGGNRRNFKVM 201
                       167 (R-P)                        196 (R-P)

Cameroon    DFANKHHLGNPVAGNFFQAKHED* 251
            |||||||||||||||||||||||
Guatemala   DFANKHHLGNPVAGNFFQAKHED* 251
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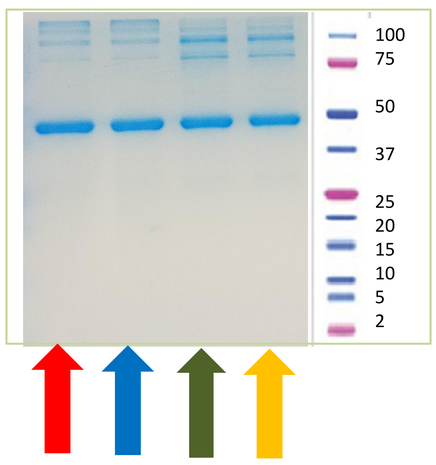
Figure 1: Amino Acid Sequence showing Mutations



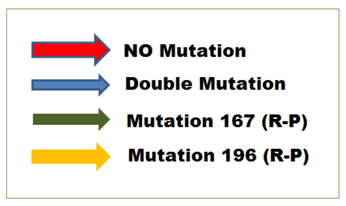
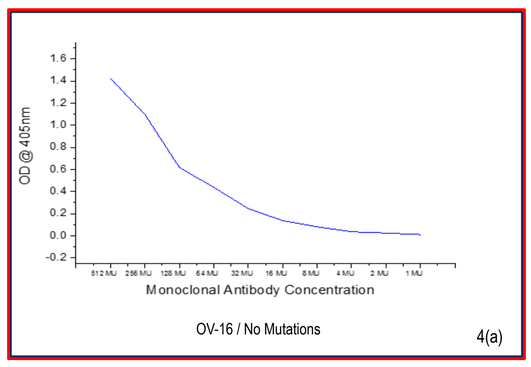
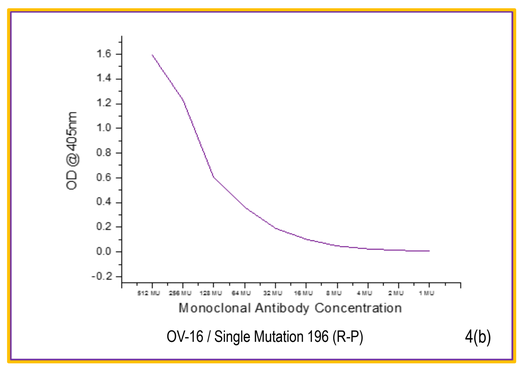
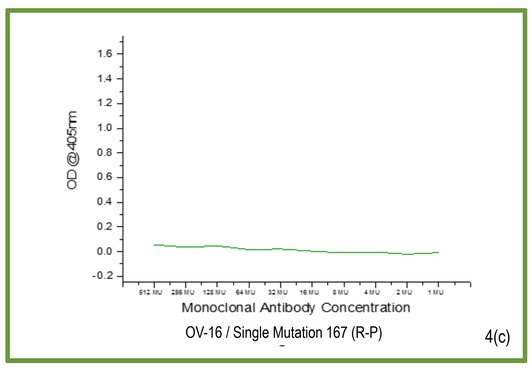


Figure 2: SDS Analysis of purified proteins







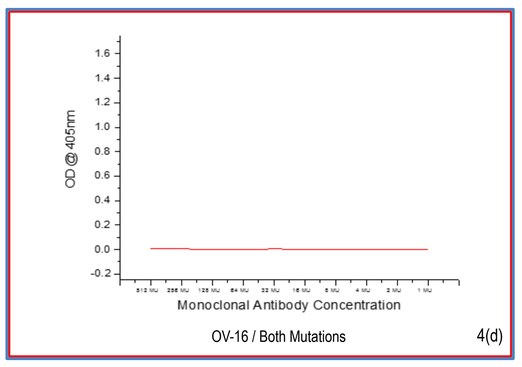
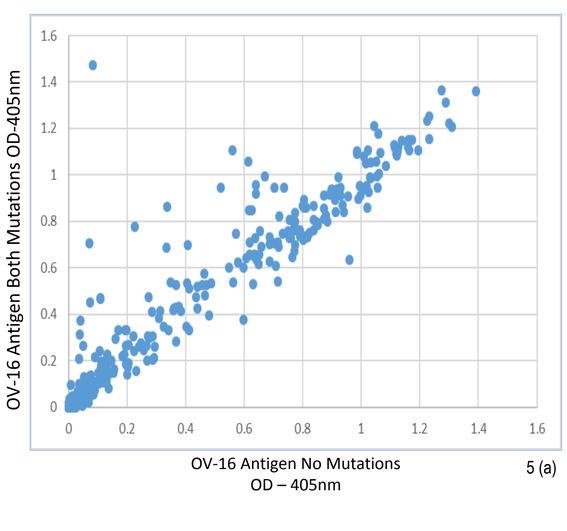
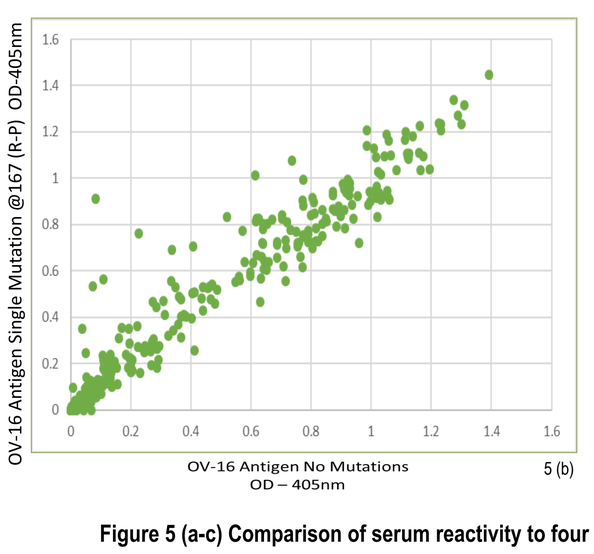
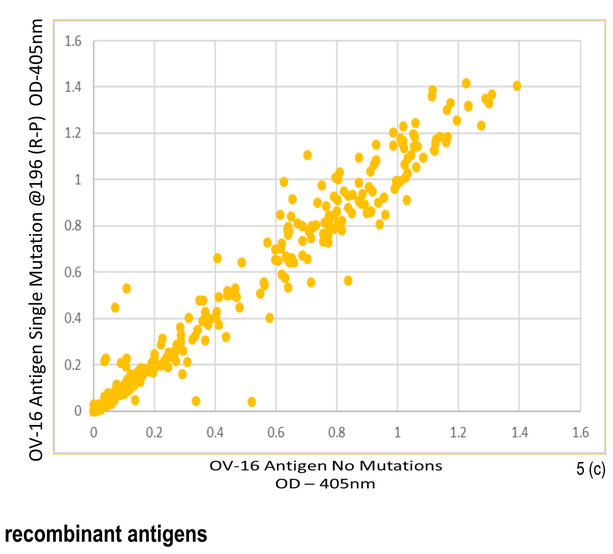


Figure 4 (a-d): Monoclonal reactivity to four recombinant antigens







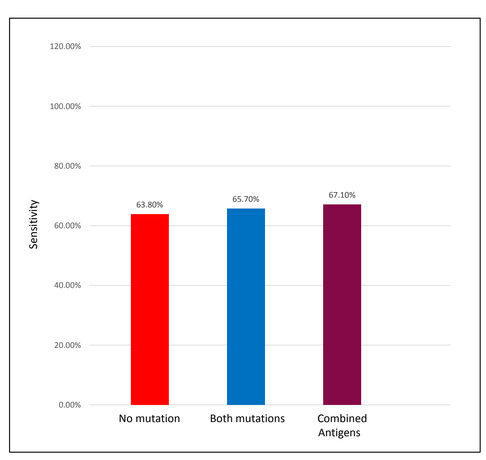


Figure 5 (a-c) Comparison of serum reactivity to four recombinant antigens

Figure 6: OV-16 ELISA Sensitivity with different antigens

CONCLUSIONS

- Polymorphisms in OV-16 antigens showed varied signaling when testing monoclonal antibodies
- Response to the four antigens generally corresponded well, indicating no major differences among antigens
- Combining the two versions of the antigen may slightly increase assay sensitivity.

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